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EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

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FeSImmChemNet

2006 - 2026
20th ANNIVERSARY
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Action Scientific Meeting 2026

FOUR YEARS OF FESIMMCHEMNET: LATEST DISCOVERIES AND THE OUTLOOK

ABSTRACT BOOKLET

CABIMER
Avenida Américo Vespucio 24, 41092 Sevilla
https://www.cabimer.es/en/home/

Kourosh.Ebrahimi@kcl.ac.uk
Ralf Wellinger: wellinger@us.es
Hélène Gaillard: gaillard@us.es

https://www.fesimmchemnet-cost.com/

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General Information

About the meeting

REGISTRATION

You are required to register on the initial day of the meeting. Throughout the meeting, attendees must sign in daily to validate their presence. These signatures serve as evidence of attendance and make you eligible for reimbursement at a later time.

INSTRUCTIONS FOR PREPARING POSTERS

All posters should be A0 size. The presenter's name should be underlined.

GUIDES TOUR

We organized a guided tour prior to the joint dinner to give you more insight into Sevillian History. It will cost about 1 to 2 Euros but is worth each Penny. Leaving from CABIMER at about 18:15-18:30h we will take a short bus ride to the meeting point at the old town at **19h**.
The meeting point is in front of the Palacio San Telmo (Plaza de Roma).
After the tour we will walk to Casa Carmen for the joint dinner at **20:30h**.

JOINT DINNER

To enhance networking and collaboration opportunities, we highly encourage your participation in our Joint Dinner.

Casa Carmen - Old Town

Calle Santander, 15, 41001 Sevilla
casacarmenrestaurant.com/restaurantes/sevilla/

The dinner will cost approximately **38 Euros** per person, unlimited free drinks (house red and white wine).

SUPPORTING CONTACTS

Dr. Kourosh Ebrahimi	Kourosh.Ebrahimi@kcl.ac.uk
Dr. Ralf Wellinger	wellinger@us.es
Dr. Hélène Gaillard	gaillard@us.es

About transportation and restaurants

GETTING FROM THE AIRPORT TO THE CITY CENTER

By Bus:
The EA Line (Airport Express) connects Seville's San Pablo Airport directly with the city center. ***Single ticket: 6 €*** *Tickets can be purchased at the airport, on board the bus, or at authorized sales points.*

By Taxi:
Taxis are located outside of the arrivals area. **Estimated Cost:** Prices are fix (independent of your destination within the city or baggage). Fares are indicated in the taxi and depend on day/hour (approximately **25 - 30 €**). You can pay cash or by credit card. The driver will print you a receipt.

By Uber or Cabify: please use the corresponding application. Outside the terminal you will find indications where the Uber/Cabify parking area is located. **Estimated Cost:** Usually cheaper than a taxi approximately **15 - 25 €** but you may have to wait 10 min till the Uber/Cabify arrives.

CITY TRANSPORTATION by TUSSAM

Bus stops: It is polite to respect a queue (ask who is last). Buses only stop if you raise hand.
Bus tickets: Enter the front door and pay cash (coins) or using the credit card (card reader). You will not get a ticket, if you pay by credit card. **Single trip: 1,40 €**

Old town to CABIMER to Old Town (5 to 10min bus drive)
Bus **C2 or 02** will take you from the **hotel Macarena to CABIMER** (bus stop named 'Facultad de comunicación). Please ask at the hotel reception for the bus stop close by.
Bus **C1** will take you from **CABIMER (hotel Cartuja) to the Old town (**bus stop 'Barqueta', 'Feria' or 'Macarena'**).**

Ask for a free city map at the hotel reception!

RESTAURANTS/FOOD

Relative to the hotels, the nearest place in the old town to have food and drinks is a neighborhood called **ALAMEDA de HERCULES**. You can check opening hours etc. using Google or Tripadvisor. Note that many restaurants are closed on Monday.

About Reimbursement

1 

Accept invitation in e-COST platform

2 

Arrange travel & accommodation

- Keep payment receipts

3 

Attend the meeting

- Sign attendance list
- Keep boarding passes & other long-distance travel tickets
- Keep receipts

4 

Submit claim via eCOST platform

- Attach any receipts related to long distance travel
- Attach boarding passes
- Attach accommodation expenses/receipt

5 

Submit the King's College claim form

- Download claim form: Events | Fesimmchemnet (fesimmchemnet-cost.com)
- Add your details and bank account associate with your COST profile
- Add your expenses as two items: (i) total travel including visa and (ii) fixed daily allowance calculated by eCOST
- The text must be clear and readable

6 

Sign and submit to Action Chair

- Email to : Kourosh.Ebrahimi@kcl.ac.uk
- Email subject should be: **COST CA21115 Claim / Belgrade 2025/ Full name**

Note:

- To be eligible for reimbursement you **must** sign attendance list for the days you attend.
- You must submit your claim within **15 days** after the meeting ends.

- Costs to be reimbursed (read carefully **COST annotated rules** for details)
  - ✓ Long-distance (>100 km) travel expenses (Receipts and travel documents (E.g. boarding passes) are required)
  - ✓ Fixed daily allowance (196 Euro/day) (Note: daily allowance is calculated by **eCOST** platform automatically based on destination and travel day and time. Please read COST annotated rules. (Accommodation receipts are required)
  - ✓ Visa (receipt is required).

Meeting programme

Day 1

02 March 2026

Morning sessions

08:30 – 09:00 — **Registration**

09:05 – 09:15 — CA Chair welcome note
Kourosh Ebrahimi | King's College London, UK

Chair: Kourosh Ebrahimi

09:15 – 10:00 — CELL-TYPE SPECIFIC IRON CONTENT REGULATION REVEALED BY SINGLE-CELL IRON QUANTIFICATION
Hal Drakesmith | University of Oxford, UK

10:10 – 10:30 — THE SUF IRON-SULFUR CLUSTER ASSEMBLY MACHINERY FROM MYCOBACTERIUM TUBERCULOSIS
Sandrine Ollagnier | CNRS, CEA, IRIG, Univ. Grenoble Alpes, France

10:40 – 11:00 — **Coffee Break** (Poster Fixing)

Chair: Peter-Leon Hagedoorn

11:05 – 11:25 — A MITOCHONDRIAL MEMBER OF THE SIDEROFLEXIN IRON-TRANSPORTER FAMILY MODULATING NEURODEGENERATION
Kostas Tokatlidis | University of Glasgow, Glasgow G12 8QQ, Scotland UK

11:30 – 11:50 — SYSTEMIC AND CELLULAR IRON DYNAMICS DURING INFECTION: INFLAMMATION-DRIVEN REDISTRIBUTION AND LIVER INTERCELLULAR COMMUNICATION
Oscar Fonseca | Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

11:55 – 12:15 — GCIS PROTEINS: A NOVEL FAMILY OF IRON-SULFUR CLUSTER-BINDING PROTEINS IN MIMIVIRUSES
Bénédicte Burlat | CNRS/AMU, Marseille, France

12:20 – 12:40 — PYRITHIONE COMPOUNDS AND THEIR INTERACTIONS WITH SELECTED FE-S CLUSTERS
Iztok Turel | University of Ljubljana, Slovenia

12:45 – 14:30 — **Lunch** (Poster Fixing)

Day 1

02 March 2026

Afternoon sessions

Chair: Bénédicte Burlat

14:30 – 15:10

RESONANCE RAMAN SPECTROSCOPY, SHINING LIGHT ON FE-S CLUSTER CONTAINING PROTEINS
Smilja Todorovic | ITQB NOVA, Universidade NOVA de Lisboa, Portugal

15:20 – 15:40

HYDROGEN SULFIDE AMPLIFIES SELENIUM-INDUCED TOXICITY IN YEAST AND HUMAN
Hélène Gaillard | University of Seville-CABIMER, Spain

15:45 – 16:05

NEW FINDINGS ON FE/S PROTEINS IN RIBOSOME BIOGENESIS
Antonio Pierik | Dept. of Chemistry, RPTU Kaiserslautern-Landau, Germany

16:10 – 16:40

Coffee Break (Poster Fixing)

Chair: Maria Andrea Mroginski

16:45 – 17:05

MITOCHONDRIALLY TARGETED IRON CHELATORS AS A POTENTIAL PHARMACEUTICAL FOR PROTOZOAN PARASITES AND PATHOGENIC YEASTS
Robert Sutak | Charles University, Czech Republic

17:10 – 17:30

EXPLORING IRON-SULFUR CLUSTERS IN METALLOTHIONEINS
Jana Gabriella Stein | University of Zurich, Switzerland

17:35 – 19:35

Poster session

Day 2

03 March 2026

Morning sessions

Chair: Ralf Wellinger

09:00 – 09:40

ENGINEERING OF NITROGENASE COFACTOR BIOSYNTHESIS IN YEAST MITOCHONDRIA
Luis Rubio | Centro de Biotecnología y Genómica de Plantas, Spain

09:50 – 10:10

MACROPHAGE FERROMETABOLISM; AN ALLY OR ADVERSARY FOR RESPIRATORY HEALTH?
Suzane Cloonan | School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland

10:15 – 10:35

EXPLORING THE MECHANISM OF METALLOENZYMES USING MICROSECOND TIMESCALE RAPID MIXING TECHNIQUES
Peter-Leon Hagedoorn | Delft University of Technology, The Netherlands

10:40 – 11:10 — Coffee Break

Chair: Susanne Kassube

11:15 – 11:35

SINGLE CELL ASSESSMENT OF IRON CONTENT IN PRIMARY HUMAN T CELLS USING LA-ICP-MS
Anna Schurich | King's College London, UK

11:40 – 12:00

A CONNECTION BETWEEN TWO ANCIENT AND ESSENTIAL CELLULAR PROCESSES, IRON-SULFUR PROTEIN BIOGENESIS AND FATTY ACID SYNTHESIS, IN ESCHERICHIA COLI
Sarah Dubrac | Institut Pasteur, France.

12:05 – 12:25

VERSATILE TRAITS OF GREEN MICROALGAE IN SEQUESTRATING AN EXCESS OF ENVIRONMENTAL MANGANESE
Ivan Spasojevic | University of Belgrade, Serbia

12:30 – 14:00 — Lunch

Day 2

03 March 2025

Afternoon sessions

Chair: Simone Ciofi-Baffoni

14:00 – 14:20	**MANGANESE IS A POTENTIAL MODULATOR OF FE/S HOMEOSTASIS** **Ralf Wellinger | Universidad de Sevilla, Spain**
14:25 – 14:45	**FE-S CLUSTER BIOGENESIS IN E. COLI** **Beatrice Py| CNRS, France**
14:50 – 15:10	**SEEING THE UNSEEN: 13C-DETECTED STRATEGIES FOR IRON-SULFUR PROTEIN STRUCTURAL ANALYSIS** **Leonardo Querci | CERM, Italy**
15:15 – 15:45	**Coffee Break**
15:50 – 17:00	**WG1-5 discussions**
17:15 – 17:45	**Action Chair, closing statement & quick guide to submit reimbursement claims**
19.00 – 20:30	**Guided tour (we leave at 18:15 from CABIMER)**
20:30	**Dinner**

Day 3

04 March 2026

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CELL-TYPE SPECIFIC IRON CONTENT REGULATION REVEALED BY SINGLE-CELL IRON QUANTIFICATION

Hal Drakesmith[1]

1 Medical Research Council Translational Immune Discovery Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DS, UK

alexander.drakesmith@imm.ox.ac.uk

Iron is crucial for cellular metabolism and cell growth, and influences all Kingdoms of life ranging from human health to marine productivity. How cellular iron accumulation varies quantitatively depending upon environmental or systemic iron availability is poorly characterized. For example, in humans, both iron deficiency and disorders of iron overload are widespread. Iron is crucial for immune responses and pathogen growth, and altered iron availability can strongly impact on the outcomes of infection and vaccination. Nevertheless, how changes in iron availability actually influence cellular iron content and function across different cell types is unknown.

To begin to investigate this problem, we developed a method to quantify metals in hundreds of cells per minute via single-cell inductively-coupled plasma mass spectrometry (sc-ICP-MS), and used this to explore iron usage by immune cells *in vitro* and *ex vivo*. Activated murine T-cells exposed to a 625-fold titration of extracellular ^{56}Fe iron maintained remarkably close homeostatic control, with iron (^{56}Fe) content varying by ~20%. However, these variations strongly correlated with cellular activation characteristics and related to proliferation. Running sc-ICP-MS downstream of flow cytometric sorting showed that murine T-cells and B-cells *ex vivo* exhibit similar mean and heterogeneity of cellular iron while splenic macrophages contain twice as much iron and more heterogeneous cell-to-cell iron content. Activated human B-cells contain ~10-fold more iron per cell than murine B-cells. We have further extended the method to detect stable low-abundance iron isotopes ^{54}Fe and ^{58}Fe. By supplying these defined isotopes as the only available sources of iron, we tracked kinetics of iron uptake into cells over time as the cell culture undergoes rounds of division.

Our data indicate that mechanisms of cellular iron homeostasis impart specific ranges and set-points of iron content to different cell types and activation states, and that small changes in iron content have large effects on cell behaviour including proliferation. Coupling single-cell iron quantification to functional analyses suggests new approaches to studying biometals applicable to cell biology, medical research and environmental science.

THE SUF IRON-SULFUR CLUSTER ASSEMBLY MACHINERY FROM *MYCOBACTERIUM TUBERCULOSIS*

Ollagnier Sandrine[a], Mohamad Hamie[a], Ingie Elchennawi[a], Christelle Caux[a], Bénédicte Burlat[b]

[a] CNRS, CEA, IRIG, Laboratoire de Chimie et Biologie des Métaux, Univ. Grenoble Alpes, Grenoble, France; [b] Aix-Marseille Université, CNRS, Bioénergétique et Ingénierie des Protéines (BIP), IMM FR3479, IM2B, Marseille 13402, France

sollagnier@cea.fr

Iron-sulfur clusters (Fe-S) are among the most ancient and versatile inorganic cofactors in nature, playing a crucial role in fundamental biological processes. Various multi-protein machineries (NIF, ISC, SUF, MIS, and SMS) mediate Fe-S cluster biogenesis across archaea, bacteria, parasites, plants, and humans [1-2]. The SUF system is nearly ubiquitous in nature [3]. It plays a general role in many bacteria and is essential for the viability of organisms lacking the ISC or NIF systems, such as *Mycobacterium tuberculosis*, *Bacillus subtilis*, *Synechocystis*, and *Staphylococcus aureus* [4-7]. We have been studying the *Escherichia coli* SUF system for 20 years, and more recently, we expanded our research to the SUF system from the pathogen *M. tuberculosis* [8]. Exciting results, particularly regarding the Fe-S scaffold $SufBDC_2$, will be presented.

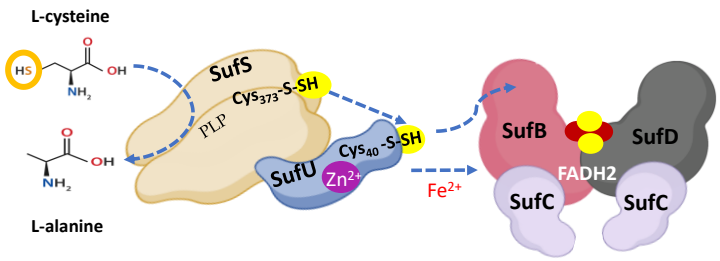

Figure 1: *M. tuberculosis* SUF system. SufS, a PLP dependent cysteine desulfurase, provides sulfur from L-cysteine. Sulfur captured by SufS is subsequently transferred to SufU, a Zn^{2+} dependent sulfurtransferase, which shuttles it to the $SufBC_2D$ complex scaffold upon which Fe-S clusters are built. A flavin cofactor, whose function is unknown, binds to the $SufBDC_2$ complex.

[1]- Lill R (2009) *Nature* 460:831–838.
[2]- Py B, Moreau PL, Barras F (2011) *Curr. Opin. Microbiol*. 14: 218-223.
[3]- Takahashi, Y and Tokumoto, U. (2002) *J. Biol. Chem*. 277, 28380-28383.
[4]- Albrecht AG, Netz DJA, Miethke M, Pierik AJ, Burghaus O, Peuckert F, et *al.* (2010) *J Bacteriol*;192:1643e51.
[5]- Huet G, Daffé M, Saves I. (2005) *J Bacteriol*; 187:6137e46.
[6]- Balasubramanian R, Shen G, Bryant DA, Golbeck JH. (2006) *J Bacteriol*; 188:3182e91.
[7]- Roberts CA, Al-Tameemi HM, Mashruwala AA, Rosario-Cruz Z, Chauhan U, Sause WE, et *al.* (2017) *Infect Immun*; 85(6). pii: e00100-17
[8]- Elchennawi I, Carpentier P, Caux C, Ponge M, Ollagnier de Choudens S. (2023) *Biomolecules*, 13 (5), 73

A MITOCHONDRIAL MEMBER OF THE SIDEROFLEXIN IRON-TRANSPORTER FAMILY MODULATING NEURODEGENERATION

Daniel Siciarz[1], Tora Gulstad[1], Erik Lacko[1], Julia Dunlop[1], Kostas Tokatlidis[1,*]

[1] School of Molecular Biosciences, Mitochondrial Biology Group, University of Glasgow, Glasgow G12 8QQ, Scotland UK

** Corresponding author*

E-mail: Kostas.tokatlidis@glasgow.ac.uk

The protein Sideroflexin 3 (SFXN3) is a mitochondrial transporter embedded in the mitochondrial inner membrane that is part of the sideroflexin family containing 5 proteins in human cells and a single isoform called Fsf1 in *S. cerevisae* [1]. Previous work [2] has shown that SFXN3 is a neuronally enriched protein expressed in synaptic terminals and regulated by key synaptic proteins, including a-synuclein. SFXN3 uses the carrier import pathway for insertion although it does not share the typical 6TMD of the standard substrates like the ADP-ATP transporter recognized by this pathway [3]. SFXN3 influences pathways associated with neurodegeneration (including Parkinson's disease and Alzheimer's disease). As a means to gain further insight in its function and interactors we knocked out SFXN3 using CRISP-R approaches in neuronal-like cell line SH-SY5Y and analysed its respiratory capacity, ATP levels, inner membrane potential and a number of other mitochondrial and metabolic alterations, suggesting a role in Fe-S biogenesis. We additionally performed similar experiments in *S cerevisiae* targeting the only SFXN homologue (the Fsf1 gene) in yeast. We will present our findings on the broad functional properties of this poorly characterized class of mitochondrial transporters that provide some links between iron metabolism and neurodegeneration.

[1] Katsafadou AI, Nebert DW, Krupenko SA, Thompson DC, Vasiliou V (2025) Update of the sideroflexin (SLC56) gene family. Hum Genomics. 2025 Jun 20;19(1):69. doi: 10.1186/s40246-025-00779-w. PMID: 40542427

[2] Ledahawsky LM, Terzenidou ME, Edwards R, Kline RA, Graham LC, Eaton SL, van der Hoorn D, Chaytow H, Huang Y-T, Groen EJN, Motyl AAL, Lamont DJ, Tokatlidis K, Wishart TM, Gillingwater TH (2022) Regulation of neurodegeneration pathways by the mitochondrial protein Sideroflexin3 (SFXN3) FEBS Journal 289(13):3894-3914 doi: 10.1111/febs.16377

[3] Functional reconstitution of the import of the yeast ADP/ATP carrier mediated by the TIM10 complex.

Luciano P, Vial S, Vergnolle MA, Dyall SD, Robinson DR, Tokatlidis K. (2001) EMBO J. 20(15):4099-106. doi: 10.1093/emboj/20.15.4099. PMID: 11483513

SYSTEMIC AND CELLULAR IRON DYNAMICS DURING INFECTION: INFLAMMATION-DRIVEN REDISTRIBUTION AND LIVER INTERCELLULAR COMMUNICATION

Óscar Fonseca[1,2], Frederico Silva[1], André Silva[3], Tânia Silva[1,4], Paulo J. Oliveira[5,6], Ana C. Moreira[1], M. Salomé Gomes[1,4]

[1]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal – Rua Alfredo Allen, 208, 4200-135 Porto, Portugal; [2]Programa Doutoral em Biologia Molecular e Celular (MCBiology), Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; [3]LAQV-REQUIMTE – Laboratório Associado para a Química Verde, Porto, Portugal; [4]ICBAS- Instituto de Ciências Biomédicas, Universidade do Porto, Portugal; [5]CNC-UC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; [6]CIBB – Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

ofonseca@i3s.up.pt

Iron metabolism is tightly regulated at both systemic and cellular levels to ensure adequate availability while preventing toxicity. During infection, these regulatory mechanisms are profoundly altered, impacting iron distribution across tissues and between cell types.

In circulation, iron is safely bound to transferrin, the main iron transporter, that carries the metal to target organs. The liver holds the largest body iron reserves and produces key iron-regulatory proteins, such as transferrin, hepcidin, and ferritin. Hepatocytes are the main responsible for the production of these proteins and for storing iron inside ferritin nanocages [1]. But other liver cell-types participate in the multiple functions of this organ, including iron homeostasis and immune response [2]. Macrophages are particularly important, as they can recycle iron from old erythrocytes and also produce regulatory proteins. In particular, during infection, macrophages produce several cytokines that may contribute to alterations in liver iron distribution. However, the mechanisms of cell-to-cell communication in the liver underlying infection-driven iron re-distribution, remain unclear [3]. The aim of this work was to elucidate them.

For a systemic approach, we resorted to a murine model of chronic *Mycobacterium avium* infection. We found that the inflammatory response of the host leads to an iron-shift from transferrin to other serum proteins, emphasizing the emergence of alternative iron-binding pathways under pathological conditions. This shift was delayed in TNFa deficient mice, suggesting a central role for this cytokine in triggering systemic iron redistribution. Successive steps of chromatographic separations of serum proteins, followed by mass spectrometry analysis, revealed haptoglobin as a mediator of infection-driven shift in iron transport.

To explore iron-related alterations caused by infection at a cellular level, we optimized an in vitro co-culture model of hepatocytes and macrophages. Hepatocytes were cultured with conditioned media collected from non-infected or infected macrophages, and the expression of key iron-related genes, such as ferritin, ferroportin and hepcidin was assessed.

Together, these complementary approaches reveal that infection-driven inflammation orchestrates iron redistribution across systemic and cellular scales. The integration of in vivo and in vitro data highlights novel mediators of iron transport and communication, providing deeper insight into the crosstalk between immunity and iron homeostasis, and offering new perspectives for targeting iron regulation during infectious and inflammatory diseases.

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GciS PROTEINS: A NOVEL FAMILY OF IRON-SULFUR CLUSTER-BINDING PROTEINS IN *MIMIVIRUSES*

Gaspard Toulis[1], Ilenia Serra[1], Audrey Lartigue[2], Martin Clémancey[3], Bruno Guigliarelli[1], Smilja Todorovic[4], Geneviève Blondin[3], Béatrice Py[5], Chantal Abergel[2], Bénédicte Burlat[1]

[1] Laboratoire de Bioénergétique et Ingénierie des Protéines, CNRS/AMU, Marseille, France
[2] Laboratoire Information Génomique et Structurale, CNRS/AMU, Marseille, France
[3] Laboratoire de Chimie et Biologie des Métaux, CEA/CNRS/UGA, Grenoble, France
[4] ITBQ Nova, Lisboa, Portugal
[5] Laboratoire de Chimie Bactérienne, CNRS/AMU, Marseille, France

bburlat@imm.cnrs.fr

Iron–sulfur (Fe–S) clusters are ancient and ubiquitous cofactors, present across the tree of life, including viruses. [1] In giant *Mimiviridae* viruses, we identified a novel family of small, atypical Fe–S cluster–binding proteins with low-complexity, glycine- and cysteine-rich sequences, named GciS (Glycine/Cysteine-rich Iron–Sulfur) proteins.[2] GciS proteins are conserved across the *Mimimegavirinae* subfamily, are among the most highly expressed genes at late stages of infection, and are highly abundant in viral particles; however, their structure and function remain unknown. Here, we present recent data and discuss potential physiological roles of GciS proteins during the viral life cycle.

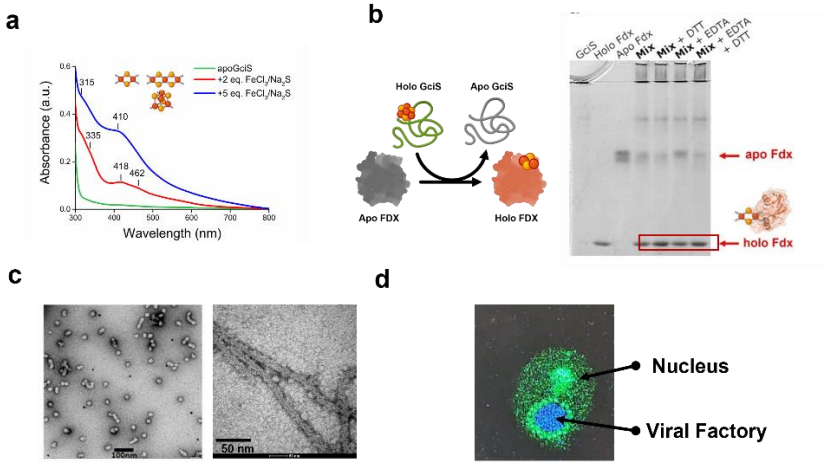

Figure 1: *In vitro,* GciS can stabilize **(a)** and exchange **(b)** various types of Fe-S clusters, using ferredoxins or monothiol glutaredoxin GrxD as model protein partners (in preparation). **(c)** NS-TEM images of recombinant purified GciS showing nanoparticles 14-20 nm in diameter that can form amyloid-like fibres. **(d)** Localization studies of *Acanthamoeba* cells transfected with the pFAST-*gciS* mimivirus mutant (8h post-infection), showing green fluorescent pFAST-GciS signals around the viral factory (nascent virions) and at the host cell nucleus.

*This work received supports from A*MIDEX (AMX-21-RID-043), French Research National Agency (ANR-25-CE11-3985), and COST Action FeSImmChemNet, CA21115. The authors thanks Drs. Marie-Pierre Golinelli-Cohen, Marianne Guiral, and Nicolas Rouhier for their gifts of purified ferredoxins and glutaredoxin.*

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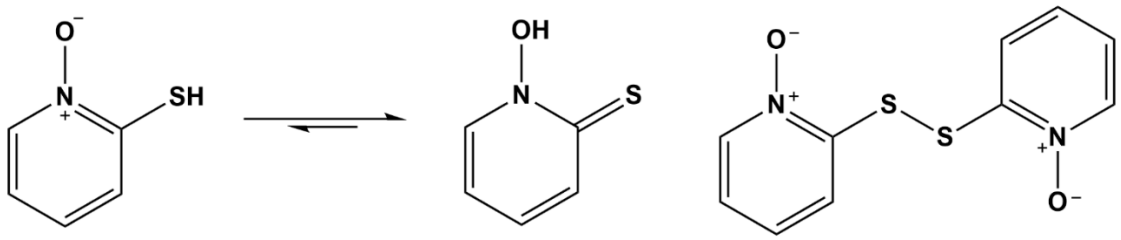
PYRITHIONE COMPOUNDS AND THEIR INTERACTIONS WITH SELECTED FE-S CLUSTERS

Uroš Rapuš[1], Mara Hebeis[2], Ingrid Span[2], Iztok Turel[1,*]

[1]*Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia*
[2]*Bioinorganic Chemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg, Egerlandstr. 1, 91058 Erlangen, Germany*

E-mail of presenting author: iztok.turel@fkkt.uni-lj.si

Pyrithione is a natural compound found in the Persian shallot. Two tautomeric forms are possible, the major form is the thione, 1-hydroxy-2(1H)-pyridinethione, and the minor form is the thiol, 2-mercaptopyridine N-oxide. It can also be readilly transformed into the disulphide form in nature or in the laboratory (see Scheme below).



Pyrithione is a known ionophore that easily forms complexes with metal ions. The most well-known complex is zinc pyrithione, an established antimicrobial agent that has been used in commercial shampoos for dandruff treatment. Zinc pyrithione exerts antifungal activity by damaging iron-sulphur clusters [1–4]. We have prepared a range of metal complexes (Cu, Zn, Ru, Re, Pt, etc.) with pyrithione and its analogues, and we are interested in their interactions with biologically important molecules [5–7]. Within this COST project, some preliminary experiments on the interaction of FDX2 (and some other proteins containing Fe-S clusters) with selected ruthenium complexes were planned in cooperation with the group of Prof. S. Ciofi (University of Florence). Unfortunately, the complexes were not sufficiently soluble to allow complete NMR experiments and ESI MS experiments were also unsuccessful due to the instability of the systems. Currently, interactions between metal pyrithiones and various pyrithiones with the IspH enzyme are being studied in cooperation with the group of Prof. I. Span. IspH is an iron-sulphur cluster-containing enzyme and is a vital drug target for bacteria and parasites such as malaria, as it is absent in humans. Crystallisation experiments are in progress.

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RESONANCE RAMAN SPECTROSCOPY, SHINING LIGHT ON FE-S CLUSTER CONTAINING PROTEINS

Smilja Todorovic[1], Diogo Agostinho[1], Célia M. Silveira[1], Sofia Pauleta[2], Elin Moe[1]

[1]*ITQB NOVA, Universidade NOVA de Lisboa, Av. da República, 2780-157 Oeiras, Portugal*

[2]*DQ, NOVA FCT, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal*

smilja@itqb.unl.pt

Upon excitation into S → Fe ligand to metal charge transfer band, resonance Raman (RR) spectra of Fe-S containing proteins enable discrimination among different cluster types, coordination and bridging vs terminal Fe-S vibrational modes [1]. RR spectroscopy has therefore played a central role in studies of Fe-S proteins with diverse structures and functions, as well as in investigations of the processes that involve these proteins [1].

Here, we demonstrate how RR, coupled with surface enhanced vibrational spectroscopy and electrochemistry, helped us elucidate the properties of Fe-S clusters in DNA glycosylases and gain deeper insight into their roles. These DNA repair enzymes operate at the forefront of the cellular response to oxidative DNA damage, recognizing the aberrant nucleoside lesion and catalyzing its removal at the onset of BER pathway [2, 3]. DNA glycosylases are present in bacteria, yeasts and humans, making them promising targets for drug development. By studying both full-length and truncated enzymes from different sources, we show the importance of i) molecular interactions in governing the redox chemistry of the Fe-S cluster and ii) employing full-length enzymes in mechanistic investigations.

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HYDROGEN SULFIDE AMPLIFIES SELENIUM-INDUCED TOXICITY IN YEAST AND HUMAN

Inés García de Oya[1,2], Iria Domínguez García[1,2], Luis C. Romero[3], Rocío Domínguez Sierra[1,2], Hayat Gahete Heluani[1,2], Cecilia Gotor[3], Néstor García-Rodríguez[1,2], Ralf E. Wellinger[1,2] and Hélène Gaillard[1,2].

[1] Centro Andaluz de Biología Molecular y Medicina Regenerativa-CABIMER, Universidad de Sevilla, Seville, Spain; [2] Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Seville, Spain; [3] Instituto de Bioquímica Vegetal y Fotosíntesis, Centro de Investigaciones Científicas Isla de la Cartuja, Consejo Superior de Investigaciones Cientificas and Universidad de Sevilla, Avda Américo Vespucio s/n, 41092 Sevilla, Spain.

gaillard@us.es

Selenium (Se) is an essential micronutrient that is metabolized as selenocysteine and is important for the function of so-called selenoproteins, which play an important role in the defense against oxidative stress. However, the cellular uptake and metabolism of Se include the formation of inorganic species, such as selenite and selenide, which are highly toxic to mammals. The boundary between protective and toxic Se levels is extremely narrow, and the molecular mechanisms underlying Se toxicity remain largely unknown. There is surprisingly little known about the intracellular interplay between selenite and endogenous metabolites such as hydrogen sulfide (H_2S), which is a gaseous signaling molecule involved in mitochondrial function, human disease, cancer and ageing. Here, we present genetic and molecular evidence showing that H_2S strongly enhances selenite-induced DNA breakage and triggers the activation of the DNA damage and oxidative stress responses in yeast and human cells. The amplification of selenite toxicity was not limited to endogenous H_2S, as the same effect was observed when cells were supplied with exogenous H_2S donors. Furthermore, H_2S was found to be critical for the conversion of selenite into hydrogen selenide (H_2Se), a physiological but highly toxic Se intermediate. Our findings reveal a novel role for H_2S in the dual impact of dietary Se supplementation on human health and opens the possibility that H_2S could act as a Trojan horse in the treatment of tumors with elevated H_2S levels using selenite.

NEW FINDINGS ON Fe/S PROTEINS IN RIBOSOME BIOGENESIS

Nadine Duppe[1], Lukas Knauer[1,2], Marc Hagebölling[1], Lena Langner[1], Martin Stümpfig[3], Volker Schünemann[2], Antonio J. Pierik[1], Daili J. Netz[1]

[1] Dept. of Chemistry, RPTU Kaiserslautern-Landau, Germany; [2] Dept. of Physics, RPTU Kaiserslautern-Landau, Germany; [3] Institut für Zytobiologie und Zytopathologie, Fachbereich Medizin and Synmikro, Philipps-Universität Marburg, Germany

pierik@rptu.de & dnetz@rptu.de

Mak16 and its interacting partner Rpf1 play a critical role at an early step in the maturation of the ribosomal 60S subunit of eukaryotes, as revealed by cryoelectron microscopy structures [1]. While these studies suggested no metal participation or the presence of a Zn^{2+} ion in Mak16, we identify a previously unexplored iron–sulfur (Fe/S) cluster in yeast Mak16 through both *in vivo* and *in vitro* methods [2]. We demonstrate a functional link between mitochondrial and cytosolic Fe/S protein biogenesis and ribosome assembly, highlighting an overlooked aspect of 60S ribosomal biogenesis. Characterization of human and yeast Mak16 revealed a redox-active $[4Fe\text{-}4S]^{2+/1+}$ cluster with a midpoint potential below –500 mV. Oxidative stress destabilizes Mak16 and disrupts its interaction with Rpf1 *in vivo*, while *in vitro* H_2O_2 causes $[3Fe\text{-}4S]^{1+}$ cluster formation. Our findings also reveal that upon binding to rRNA expansion segment 7 the redox properties of the nearby Fe/S cluster largely remain unchanged. However, disruption of Fe/S cluster coordination destabilized Mak16, impaired the Mak16–Rpf1 complex formation and decreased the 25S rRNA level. The critical role of Fe/S proteins in eukaryotic DNA replication [3] and repair, mitoribosomal function [4,5], and maturation [6] has now been extended to nuclear ribosomal assembly [2]. Relying on a vulnerable cofactor comes at a cost, as cluster loss can severely disrupt essential cellular processes. The inherent biosynthetic complexity and instability of the Fe/S cluster of Mak16 allows it to function as sensor for redox imbalance, creating the possibility to regulate cellular homeostasis under stress.

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MITOCHONDRIALLY TARGETED IRON CHELATORS AS A POTENTIAL PHARMACEUTICAL FOR PROTOZOAN PARASITES AND PATHOGENIC YEASTS

Robert Sutak[1]

[1] *Department of Parasitology, Faculty of Science, Charles University, BIOCEV, Vestec 25250, Czech Republic*

sutak@natur.cuni.cz

Iron is a micronutrient that is both essential and potentially toxic, making it a promising target for antimicrobial intervention. As part of nutritional immunity, the host restricts iron availability, to inhibit parasitic growth, while the parasites and fungi deploy efficient iron-scavenging strategies. Because mitochondria are the cellular hub of iron metabolism, mitochondrial targeting of iron chelators represents an attractive therapeutic approach.

We evaluated mitochondrially targeted derivatives of the clinically approved iron chelators deferoxamine (DFO) and deferasirox (DFX), generated via conjugation to the lipophilic triphenylphosphonium (TPP) cation. These compounds exhibit potent activity against a broad spectrum of pathogens, including Trypanosoma spp., Toxoplasma gondii, Leishmania spp., Plasmodium falciparum, pathogenic yeasts. Both mitochelators show remarkable selectivity and benefits from prior development as a promising anticancer agent, highlighting opportunities for drug repurposing.

Structure–activity relationship studies and mechanistic analyses demonstrate that mitochondrial accumulation, rather than iron chelation alone, is the primary driver of antimicrobial activity. In both protozoan parasites and pathogenic yeasts, mitochelators disrupt mitochondrial membrane potential with minimal global iron deprivation.

EXPLORING IRON-SULFUR CLUSTERS IN METALLOTHIONEINS

Jana Gabriella Stein[a], Monica Perinelli[a], Eva Freisinger[a]

[a] *University of Zurich, Department of Chemistry, Winterthurerstrasse 190, 8057 Zurich, Switzerland*

jana.stein@chem.uzh.ch

Iron-sulfur (Fe-S) cluster proteins are ubiquitously found in all life forms, exhibiting diverse functions as electron carriers, enzymatic catalysts, and gene regulators. Typically, these proteins bind [2Fe-2S], [3Fe-4S], or [4Fe-4S] core units through cysteine thiolate groups, although additional coordination by histidine has also been observed.

Metallothioneins (MTs) represent another widely distributed protein family characterized by a high cysteine content and pronounced metal-binding capacity. In plants, MTs are primarily associated with Zn^{II} and Cu^{I} homeostasis, detoxification of Cd^{II} or Hg^{II}, and protection against oxidative stress. Plant MT subfamilies MT1-3 share common structural features, such as two cysteine-rich domains connected by a Cys-free linker region. Ordered secondary structures in apo-MTs are rarely observed, and their conformation is largely dictated by metal coordination, which, similar to Fe-S cluster proteins, involves cysteine thiolate ligands.

Until now, research on MTs has predominantly focused on their ability to form metal-thiolate clusters with d^{10} metal ions such as Zn^{II} or Cd^{II}. Reports on iron coordinating to MTs are rare [1-3]. A former PhD student in our group recently found a Fe-S cluster associated with a plant MT3 protein during heterologous expression in *E. coli*. This unexpected finding raises fundamental questions about the metal-binding versatility and potential physiological functions of plant MTs.

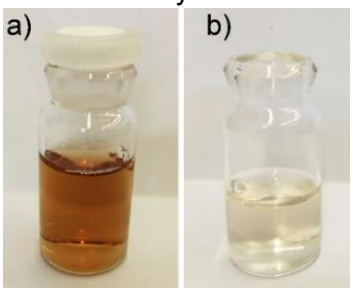

Figure 1. a) Unusual color of mal_MT3 compared to b) a non-iron binding plant MT.

Building on this observation, I optimized established methodologies from the Fe-S protein field for application to the metallothionein family, focusing on MT3 from *Malus domestica*, mal_MT3. *In vitro* reconstitution and spectroscopic characterization of the cluster-bound protein shows structural similarities to the Dre2 protein family, which is known to have the ability to act as an Fe-S cluster acceptor protein [4-5]. Motivated by these parallels, I investigated whether mal_MT3 can function in a similar way, using the NEET protein from *Arabidopsis thaliana* as a potential donor. This protein is well known for its cluster donor ability to a suitable acceptor protein due to its unusual cluster coordination involving three cysteines and one histidine. Preliminary results supporting this transfer will be presented in this talk.

Taken together, these findings raise the question if plant MTs might possess previously unrecognized Fe–S cluster-related functions, expanding their known biological roles beyond d^{10} metal homeostasis.

Project funding from the Swiss National Science Foundation (SNSF), the Science Faculty of UZH, and the University of Zurich is gratefully acknowledged.

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ENGINEERING OF NITROGENASE COFACTOR BIOSYNTHESIS IN YEAST MITOCHONDRIA

Luis M. Rubio[1], Stefan Burén[1], Katarzyna Dobrzynska[1], Lucía Payá-Tormo[1], Xi Jiang[1], Carlos Echavarri-Erasun[1]

[1] Centro de Biotecnología y Genómica de Plantas (UPM-INIA/CSIC), Campus Montegancedo, 28223 Pozuelo de Alarcón (Madrid), Spain

E-mail: luis.rubio@csic.es

The biosynthesis of the iron-molybdenum cofactor (FeMo-co) for Mo-nitrogenase depends on a sophisticated interplay between various nitrogen fixation (*nif*) gene products [1]. In this process, the SAM radical enzyme NifB, the scaffold protein NifEN, and the dinitrogenase reductase NifH are essential. NifB initiates the process by employing radical chemistry to fuse two [4Fe-4S] clusters and extract C from S-adenosylmethionine (SAM) to form an [8Fe-9S-C] intermediate known as NifB-co. This intermediate is subsequently transferred to NifEN, where it undergoes conversion into FeMo-co through the substitution of an iron atom with molybdenum and the addition of homocitrate —a transformation that is dependent on its interaction with NifH. Here, the complete synthesis of FeMo-co is achieved using proteins expressed in the mitochondria of *Saccharomyces cerevisiae*. Instead of solely relying on proteins from traditional diazotrophic models, components from Archaea, Bacteria, and Eukarya were integrated, yielding multiple variants of NifB [2], NifH [3], and NifEN [4] that maintain their functional complexes even under aerobic yeast cultivation. These purified proteins, when combined in *in vitro* assays, successfully reconstituted an active nitrogenase enzyme. This achievement not only demonstrates the interspecies compatibility of nitrogenase components but also paves the way for future advances in engineering nitrogen fixation in eukaryotic systems, with promising implications for agricultural and biotechnological innovations.

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MACROPHAGE FERROMETABOLISM; AN ALLY OR ADVERSARY FOR RESPIRATORY HEALTH?

Suzanne M. Cloonan[a]

[a] *School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland,*

suzanne.cloonan@tcd.ie

Iron metabolism is a central determinant of macrophage function, immunometabolism, and lung tissue homeostasis. In my laboratory we investigate how iron–dependent pathways, spanning iron–sulfur cluster biogenesis, heme synthesis and turnover, ferritin regulation, and mitochondrial iron handling, shape macrophage identity and vulnerability during chronic and acute lung injury. We have shown that dysregulated mitochondrial iron loading drives oxidative stress, defective bioenergetics, and impaired mucociliary clearance in cigarette-smoke–induced COPD, and that mitochondrial iron chelation can reverse these pathogenic features. Our newest study also reveals that extracellular iron, ferritin, and host siderophores such as lipocalin-2 are linked to specific microbial signatures in the lower airways of smokers and individuals with COPD, implicating iron availability as a driver of lung microbiome composition and COPD exacerbation biology.

More recently, we identified ferritin heavy chain (FTH1) as a key regulator of macrophage susceptibility to ferroptosis, demonstrating that its loss protects macrophages during hyperoxic stress and attenuates ARDS severity. Our current work also reveals how epithelial metabolic pathways coordinate with macrophage iron handling to fine-tune lung regeneration. Together, these studies establish iron metabolism as a unifying axis connecting macrophage biology, epithelial resilience, host–pathogen interactions, and environmental injury responses. By integrating iron biology with immunometabolism, my research provides mechanistic insights and therapeutic opportunities relevant to COPD, ARDS, and broader respiratory disease contexts aligned with the Fe-S COST's collaborative, multidisciplinary mission.

EXPLORING THE MECHANISM OF METALLOENZYMES USING MICROSECOND TIMESCALE RAPID MIXING TECHNIQUES

R.A. Schmitz[a], M. Boll[b] , X. Li[c], D. Tischler[d], S. Gao[e], S. Todorovic[f], P.L. Hagedoorn[a]

[a] Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629HZ, Delft, The Netherlands; [b] Microbiology, Faculty of Biology, Albert-Ludwigs-University Freiburg, Freiburg, Germany; [c] Division of Biology and Chemistry, Paul Scherrer Institute, Villigen, Switzerland; Microbial Biotechnology, [d] Ruhr University Bochum, Bochum, Germany; [e] Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, China; [f] Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

p.l.hagedoorn@tudelft.nl

Roughly one third of all enzymes contain one or more metal cofactor, and the identity, oxidation state and coordination environment of the metal cofactor provides essential functional information. EPR spectroscopy is very useful to determine the redox state and obtain information on the direct environment of the metals sites in enzymes. We have explored the mechanism of metalloenzymes using ultrafast mixing and freezing techniques and used EPR spectroscopy to examine enzyme catalytic intermediate states of a heme enzyme chlorite dismutase and a tungsten-cofactor and FeS cluster containing enzyme Benzoyl-coA reductase [1,2]. Furthermore we have provided direct evidence on the role of a heme cofactor in an unexpected heme enzyme Styrene oxide isomerase (figure 1) and in a new catalase-family enzyme Chanoclavine synthase which exhibits an unprecedented superoxide mechanism [3,4].

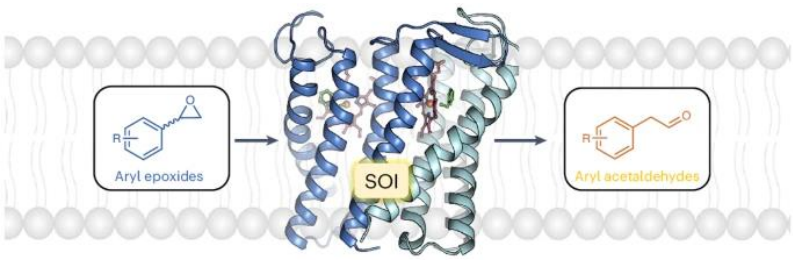

Figure 1: Styrene oxide isomerase (SOI) is a heme containing membrane bound enzyme [3]

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SINGLE CELL ASSESSMENT OF IRON CONTENT IN PRIMARY HUMAN T CELLS USING LA-ICP-MS

Diana M. Carp,[1] Piotr Golda,[2] Alexander Griffiths,[2,3] Katie R. Flaherty,[1] Alexander Morrell,[2] Anna Schurich[1,4]*

[1] *Department of Infectious Diseases, School of Immunology and Microbial Sciences, King's College London, London SE1 9RT, UK diana.carp@kcl.ac.uk and anna.schurich@kcl.ac.uk*

[2] *London Metallomics Facility, Faculty of Life Sciences & Medicine, King's College London, UK*

[3] *Department of Earth Sciences and Engineering, Imperial College London, London, UK*

[4] *Lead contact*

**Presenting author: anna.schurich@kcl.ac.uk*

Immune cells have highly specialized metabolic demands to fuel and regulate their functions. Naïve and resting T cells have a low metabolic and proliferative rate, however, upon activation T cells reprogram their metabolism to meet the increased energetic cost of proliferation and effector molecule production [1]. Transition metals, such as iron, support vital metabolic and signaling functions in immune cells and cellular iron concentrations are tightly controlled. In T cells, both iron deficiency and iron overload have been linked to immune dysfunction [2] . However, homeostatic iron concentrations in T cells, as well as the changes that occur during T cell activation, remain poorly understood due to the difficulty of accurately measuring iron content in single cells, especially in small cells like individual T cells. We use laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS) to accurately quantify the total amount of endogenous iron in individual primary human T cells. Our technique allows for targeted selection of single cells and reproducible quantification of iron at femtogram level. Our findings reveal that iron levels in resting T cells were similar in all human donors assessed. In contrast, T cell activation led to diverse patterns of iron content between individual cells and between donors, indicating specialized needs during cell differentiation. Our method enables future detailed studies into the biological role of iron in cellular metabolism and function and might reveal novel targets for therapeutic intervention.

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A CONNECTION BETWEEN TWO ANCIENT AND ESSENTIAL CELLULAR PROCESSES, IRON-SULFUR PROTEIN BIOGENESIS AND FATTY ACID SYNTHESIS, IN *ESCHERICHIA COLI*

Soufyan Fakroun[1], Guillaume Bouvier[2], Marouane Libiad[1], Emmanuel Sechet[1], Emmanuelle Bouveret[1], Frédéric Barras[1] and Sarah Dubrac[1]

[1] *Institut Pasteur, Université Paris-Cité, CNRS UMR 6047, Department of Microbiology, Stress Adaptation and Metabolism in enterobacteria Unit, Paris, France.*

[2] *Institut Pasteur, Université Paris-Cité, CNRS UMR 3528, Department of Structural Biology and Chemistry, Structural Bioinformatics Unit, Paris, France.*

sarah.dubrac@pasteur.fr

Iron-sulfur [Fe-S] clusters are ubiquitous cofactors of a wide array of structural and functional diverse proteins. Acyl Carrier Protein (ACP) is the universal factor required for fatty acid (FA) synthesis. In this study, we demonstrated that [Fe-S] and FA biosynthesis pathways are coordinated processes in *E. coli*, driven by a physical interaction between ACP and the ISC [Fe-S] biogenesis machinery. Using bacterial two-hybrid assays, co-purification and biochemical analyses, we demonstrated a molecular interaction between ACP and IscS, the ISC machinery cysteine desulfurase that provides sulfur for [Fe-S] cluster formation. Structural modeling and directed mutagenesis pinpointed the ACP-binding site in a region of IscS shared for interactions with other components of the ISC [Fe-S] biogenesis system. This led us to propose a mechanism of competition between IscS interactant that could be responsible for the regulation of its activity. At the cellular level, ACP depletion was found to disrupt ISC-dependent [Fe-S] cluster biogenesis, diminishing the activity of key [Fe-S]-dependent regulators (IscR, FNR, NsrR) and enzymes (aconitase, biotin synthase). Our findings underscore a functional link between [Fe-S] cluster biogenesis and fatty acid metabolism with far-reaching unexplored intricacies of metabolic coordination and cellular homeostasis. Comparison with eucaryotic systems highlight a strong evolutive driving force towards a link between [Fe-S] cluster and fatty acid biosynthesis in all living systems.

VERSATILE TRAITS OF GREEN MICROALGAE IN SEQUESTRATING AN EXCESS OF ENVIRONMENTAL MANGANESE

Ivan Spasojević
Group for Bioinorganic Chemistry and Redox Processes, University of Belgrade – Institute for Multidisciplinary Research, National Institute of the Republic of Serbia

Microalgae are exposed to manganese excess in metal-infested waters and have to mitigate toxic effects at least in part by accumulating Mn in the form of chelates or clusters. On the other hand, Mn is a co-factor of different catalytic centers and some microalgal species appear to be capable of opportunistic accumulation. Herein, we compared the ways that different green microalgae accumulate Mn, using synchrotron radiation-based techniques – X-ray fluorescence microscopy (XRF), X-ray absorption fine structure, and EPR spectroscopy. High sublethal concentrations of Mn(II) have been applied in the early stationary phase of growth of cultures of *Chlorella sorokiniana*, *Haematococcus pluvialis*, and *Chlamydomonas acidophila,* and *Chlamydomonas reinhardtii*. XRF microscopy generated maps of distribution of Mn and other elements within a single cell, which enabled the identification of location of Mn deposits on the subcellular level. In the early phase of the treatment, Mn was accumulated in the cell wall or mucilage. It appears that acidocalcisomes are the final destination for Mn in all analyzed strains, although deposits in other compartments are possible. In *C. sorokiniana*, Mn was accumulated in the form of a multivalent cubic Mn-O cluster. In *H. pluvialis*, Mn(II) was coordinated to sulfated polysaccharides in distorted octahedral geometry. In Chlamydomonas strains, Mn appears to be coordinated to polyphosphates mainly in Mn(II) redox form, with Mn(III) being detected in *C. acidophila*. Such versatile traits could be exploited in the biosynthesis of Mn compounds.

MANGANESE IS A POTENTIAL MODULATOR OF FE/S HOMEOSTASIS

Inés García de Oya[1,2], Manuel Peral Vázquez[1,2], Antonio Díaz Quintana[3], Hélène Gaillard[1,2]and Ralf E. Wellinger[1,2].

[1] Centro Andaluz de Biología Molecular y Medicina Regenerativa-CABIMER, Universidad de Sevilla, Seville, Spain; [2] Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Seville, Spain; [3] Instituto de Bioquímica Vegetal y Fotosíntesis, Centro de Investigaciones Científicas Isla de la Cartuja, Consejo Superior de Investigaciones Cientificas and Universidad de Sevilla, Avda Américo Vespucio s/n, 41092 Sevilla, Spain

wellinger@us.es

Manganese (Mn) is a redox metal that is essential for life. Cells use the redox potential of Mn to control the detoxification of reactive oxygen species (ROS). Mn may also alter enzymatic activities by competing with other transition metals such as magnesium (Mg) or iron (Fe). Thereby, Mn has been shown to alter DNA cell cycle regulation, polymerase and telomerase activity, and to interfere with metabolic regulation by TORC1 signaling or mitochondrial activity by coenzyme Q (CoQ).

Cellular Mn transport is highly conserved from yeast to human. In fact, it has been shown that the *ATP2C1* gene encoding the human Ca2+/Mn2+ transporter SPCA1 can complement for the function of its yeast homologue Pmr1. Mutations in hATP2C1 cause Hailey-Hailey disease, while the absence of Pmr1 renders cells highly sensitive to Mn overload. To better understand the impact of Mn overload on yeast cells, we carried out an unbiased screening for mutations that counteract the Mn hyper-sensitivity of yeast cells lacking Pmr1. Surprisingly, INDELS leading to Mn-resistance of *pmr1* mutants were restricted to only 2 genes. One of them, *FRA1*, encodes for a protein involved in the negative regulation of iron regulon transcription. We thus further explored how Fra1 is linked to Mn resistance. Our results indicate that Mn^{2+} transport can impact Fe^{2+} transport and thus Fe/S cluster homeostasis.

FE-S CLUSTER BIOGENESIS IN *E. COLI*

B. Py

Laboratoire de Chimie Bactérienne, CNRS 31 chemin Joseph Aiguier 13009 Marseille France

py@imm.cnrs.fr

This presentation will focus on the major strengths of *E. coli* for understanding conserved principles of Fe–S cluster biology, including its genetic tractability, well-defined assembly pathways and compatibility with high-resolution functional analyses. Our studies reinforce the central role of *E. coli* as a reference system to dissect the molecular mechanisms governing Fe–S cluster assembly, trafficking and regulation. In addition, studies performed in other bacterial model systems have enriched our view of Fe–S biology, notably in the regulatory circuits controlling Fe–S cluster homeostasis. These comparative approaches highlight both conserved and divergent strategies among bacteria.

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A Diverged Transcriptional Network for Usage of Two Fe-S Cluster Biogenesis Machineries in the Delta-Proteobacterium Myxococcus xanthus.

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Escherichia coli monothiol glutaredoxin GrxD replenishes Fe-S clusters to the essential ErpA A-type carrier under low iron stress.

SEEING THE UNSEEN: ^{13}C-DETECTED STRATEGIES FOR IRON-SULFUR PROTEIN STRUCTURAL ANALYSIS

Leonardo Querci[1], Deborah Grifagni[1], Marco Schiavina[1], Liza Burgassi[1], José Malanho Silva[1], Ines B. Trindade[2,3], Gabriele Palma[1], Marie-Pierre Golinelli Cohen[4], Ricardo O. Louro[2], Ravera Enrico[1], Francesca Cantini[1], Simone Ciofi-Baffoni[1], Mario Piccioli[1]

[1]European Center of Magnetic Resonance (CERM) & University of Florence, Department of Chemistry 'Ugo Schiff', Via Luigi Sacconi n. 6, 50019 Sesto Fiorentino (FI), Italy; [2] Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA), Universidade Nova de Lisboa, Av. da República (EAN), 2780-157, Oeiras, Portugal; [3] Division of Biology and Biological Engineering, California Institute of Technology, CA 91125, Pasadena, USA; [4] CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, Université Paris-Saclay, Gif-sur-Yvette, France

querci@cerm.unifi.it

Iron-sulfur (FeS) proteins are central to cellular redox processes, electron transfer, and metal homeostasis, yet their structural characterization in solution via Nuclear Magnetic Resonance spectroscopy remains challenging due to strong paramagnetic effects that limit conventional spectroscopic approaches [1]. In this work, we present a suite of integrated strategies combining advanced paramagnetic NMR methodologies with targeted biochemical and biophysical studies to elucidate structure-function relationships in biologically relevant FeS proteins [2-4]. We developed and applied ^{13}C-detected NMR approaches specifically optimized for paramagnetic systems, including relaxation-filtered experiments, measurement of paramagnetic relaxation enhancements, and pulse sequences based on optimal control theory [5]. When combined with ultra-high magnetic fields, these methods significantly extend the range of observable nuclei in the vicinity of metal centers, enabling the retrieval of structural restraints that are inaccessible with standard ^{1}H-based NMR experiments. These methodological advances were applied to mitochondrial ferredoxins and NEET family proteins, providing new insights into electron delocalization pathways and the structural response of FeS clusters to redox and nitrosative stress. Our results reveal how local structural environments near FeS clusters govern protein stability and reactivity, and how external perturbations such as nitric oxide and oxidative conditions modulate cluster integrity and function. By bridging methodological innovation with biologically relevant applications, this work demonstrates how modern paramagnetic NMR can be leveraged to probe metalloproteins at atomic resolution and offers a general framework for studying challenging paramagnetic systems in solution.

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TRANSFERRIN–TRANSFERRIN RECEPTOR AXIS LINKS IRON UPTAKE TO FE–S CLUSTER HOMEOSTASIS IN COLON CANCER

Goran Miljuš[a], Vesna Malenković[b], Blagoje Đukanović[c], Nikola Kolundžić[a], Olgica Nedić[a]

[a]*Institute for the Application of Nuclear Energy, University of Belgrade,* [b]*Clinical Medical Centre "Bežanijska Kosa", Belgrade, Serbia*

email: goranm@inep.co.rs

Iron–sulphur (Fe–S) clusters are indispensable cofactors for mitochondrial and cytosolic proteins involved in respiration, DNA maintenance, and immune signaling. Their biogenesis critically depends on iron delivered to mitochondria, where the ISC (iron–sulphur cluster) assembly machinery operates. Although the molecular components of the ISC system are well characterized, the upstream mechanisms controlling iron entry into the labile iron pool (LIP) and its subsequent availability for mitochondrial Fe–S cluster biosynthesis remain poorly defined.

Transferrin (Tf)–transferrin receptor (TfR)–mediated endocytosis represents the principal route of cellular iron acquisition and is therefore a potential regulator of iron flux toward mitochondrial Fe–S cluster assembly. Colon cancer (CC), characterized by increased iron demand and metabolic reprogramming, provides a relevant pathological context to explore alterations in this regulatory axis.

Serum and colon tissue samples from 45 CC patients and serum samples from 25 healthy controls were analyzed. Serum iron status parameters (Tf, iron, hemoglobin and total iron-binding capacity) were determined. Transferrin was isolated by immunoprecipitation and structurally characterized using immuno- and lectin-blotting to assess carbonylation and glycosylation patterns, as well as by SELDI-TOF mass spectrometry employing ion-exchange, metal affinity and hydrophobic surfaces. The Tf–TfR relationship was examined by immunoprecipitation of Tf from colon tissue using anti-TfR antibodies.

Circulating Tf concentration in CC patients was approximately two-fold lower than in healthy controls. Transferrin from CC patients exhibited increased protein carbonylation, altered glycosylation (including enhanced sialylation and Galβ4GlcNAc content), modified surface charge density, and enhanced metal-binding properties, as revealed by differential interactions with ion-exchange and metal affinity surfaces. Tissue analyses demonstrated increased membrane-associated TfR expression in cancerous compared to non-cancerous colon tissue.

Based on these findings, we propose that cancer-associated structural modifications of transferrin, together with elevated transferrin receptor expression, modulate iron influx into the labile iron pool, thereby influencing mitochondrial iron availability for ISC-mediated Fe–S cluster biosynthesis and potentially affecting Fe–S cluster homeostasis in cancer cells. Dysregulation of this upstream iron delivery pathway may not only impact Fe–S cluster assembly but could also represent a vulnerability of cancer cells, potentially linking altered mitochondrial iron handling to iron-dependent oxidative stress and ferroptosis.

VITAS ASSAY FOR IDENTIFYING ELECTRON TRANSFER PARTNER OF ANTIVIRAL RADICAL SAM ENZYME HSAND

Mengdi Wu[1], Nghi Thao Hoang[2],Deborah Grifagni[3], Meritxell Wu-Lu[4], Yujie Sheng[3], Kourosh H. Ebrahimi[1*], Molecular Requirements for Activating Antiviral Radical-Based Catalysis, manuscript in revision in Nature Communications.

[a] Institute of Pharmaceutical Science, King's College London, London, UK; [b] Department of Pharmacy, Da Nang University of Medical Technology and Pharmacy, Da Nang, Viet Nam; [c] Magnetic Resonance Center and Department of Chemistry, University of Florence, Sesto Fiorentino, Italy; [d] Department of Chemistry, Technical University of Berlin, Berlin, Germany; [e] Agilent Technologies, Cheadle, UK; [f] Department of Biotechnology, Delft University of Technology, Delft, the Netherlands

mengdi.1.wu@kcl.ac.uk

As a radical SAM enzyme, human SAND (hSAND) has a poorly characterized catalytic mechanism, particularly regarding the sources and pathways of the electron donor that transfer the electrons to activate its catalytic function. This knowledge is critical for understanding the physiological function of the enzyme. This study describes the application of the VITAS (Viral polymerase-Inhibition Toxin-Associated Selection) assay [1] in investigating the electron transfer pattern of hSAND.

The VITAS assay is a sensitive in cell selection assay to measure formation of antiviral nucleotide analogues chain-terminating viral RNA polymerases (Figure 1) . I demonstrate how the application of this assay helped discover the electron transfer partner of human antiviral enzyme hSAND and identifying the structural features important for electron transfer. These findings provide critical experimental evidence for the catalytic mechanism of hSAND and validate the unique advantages of VITAS in screening electron donors and dissecting electron transfer networks in radical SAM enzymes.

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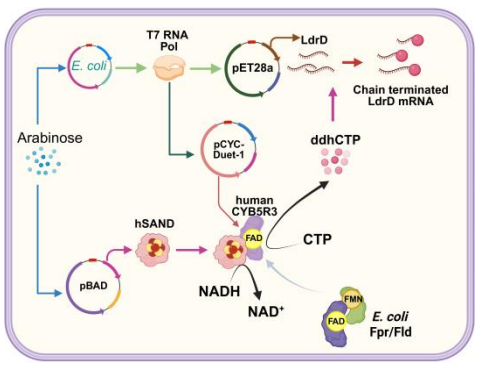

Figure 1. Principles of the VITAS assay. In an engineered E. coli, the expression of viral T7 RNA polymerase (Pol) is induced by adding the first inducer, arabinose. T7 polymerase mediates the expression of a toxin protein under the control of the leaky T7 promotor. Consequently, cell growth is blocked. When the cells are transformed with a plasmid expressing hSAND only or doule transformation with CYB5R3 and hSAND, the activity leads to the synthesis of ddhCTP in E. coli, prematurely terminating the activity of T7 polymerase and blocking the synthesis of a functional toxin. As a result, cell growth is rescued.

STRUCTURAL INSIGHTS INTO FE-S PROTEIN MATURATION

Steffen Winkler[a], Anna Runa von Wyss[a], Alexander Grunau[a], Susanne Kassube[a]

[a] *Department of Biochemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland*

s.winkler@bioc.uzh.ch

The biogenesis of cytosolic and nuclear Fe-S proteins relies on the concerted interplay of numerous factors to ensure the coordinated assembly of Fe-S clusters and their targeted insertion into apo proteins [1]. The cytosolic iron-sulfur assembly targeting complex (CTC) plays a central role in the pathway, as it recognizes apo Fe-S proteins and facilitates the transfer of Fe-S clusters to complete their maturation [2, 3]. CIAO2B, the smallest CTC subunit, was previously shown to contain a highly reactive cysteine residue and is therefore implicated in Fe-S cluster transfer [4]. The beta-propeller protein CIAO1 is additionally required for the maturation of all known client proteins [5]. Furthermore, DNA replication and DNA repair proteins require the alpha-helical repeat protein MMS19, and further adaptors are necessary for the maturation of certain client proteins [6-8]. It remains unclear why maturation of some client proteins can be accomplished by the CIAO1-CIAO2B core complex alone, whereas others require additional adaptors. Moreover, the molecular determinants that govern client-specific recognition remain poorly understood. The recent identification of potential recognition motifs, namely a C-terminal tripeptide and an internal pentapeptide [9, 10] appear insufficient to fully account for client specificity, as neither sequence uniquely identifies Fe-S proteins.

In our recent work, we used cryo-electron microscopy to determine several high-resolution structures of CTC bound to distinct Fe-S proteins. Structural analysis confirms the functional importance of a conserved peptide-binding pocket within CIAO2B and reveals additional, client-specific interaction surfaces that differ among Fe-S proteins [8]. Together, these findings provide a structural framework for understanding how the CTC achieves both universal and client-tailored recognition, underscoring the complexity of cytosolic and nuclear Fe-S protein maturation.

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STUDY OF BIOACTIVE PROPERTIES OF SELECTED TRANSITION METALS COORDINATED WITH IMINE LIGANDS

Mersiha Suljkanović[1], Jasmin Suljagić[2]

[a] *Faculty of Natural Sciences and Mathematics, Urfeta Vejzagića 4, Tuzla, Bosnia and Herzegovina;*

[b] *Faculty of Technology, Urfeta Vejzagica 8, Tuzla, Bosnia and Herzegovina*

E-mail of presenting author: mersiha.suljkanovic@untz.ba

Imine ligands are known as widely used organic compounds that can coordinate many metals (especially transition metals) to form stable metal complexes with various therapeutic applications [1,2]. Most of them show biological activities, including: antibacterial, antifungal, antidiabetic, antitumor, antiproliferative, anticarcinogenic, herbicidal, and anti-inflammatory effects [3,4]. These complexes are used as model molecules for biological oxygen transport systems [5,6]. Their application is based on the structural similarity between Schiff bases possessing different donor atoms (N, O, S, etc.) and biologically active compounds found in natural biological systems [7,8].

In this work, complexes of selected transition metals (Cu, Co, Ni and Fe) with ninhydrin-based imines and amino acids methionine and cysteine were synthesized, characterized and their antioxidant activities and antimicrobial properties were studied *in vitro*.

Different structure of complexes resulted in a higher antioxidant activity of the methionine complexes, compared to cysteine complexes. For the tested bacterial strains, the greatest inhibition resulted from Ni(II) complex with cysteine; followed by Co(II) complex with cysteine and Cu(II) with methionine.

Keywords: Imine ligands, transition metals, antioxidant activity, antimicrobial activity.

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IRON ACCUMULATION WITHOUT MINERALIZATION: INTERCONNECTION WITH DISRUPTED FES CLUSTER HOMEOSTASIS IN CELLULAR NEURODEGENERATION

J. Vojtova[1], K. Dibdiakova[1], M. Pokusa[1], M. Liskova[1], M. Brodnanova[1], M. Skratek[2], D.Soloviov[3], E. Baranovicova[1], O. Tomchuk[4], E. Cizmar[5], D. Volavka[5], O. Strbak[1*]
[1]Biomedical Centre Martin, JFM CU, Mala Hora 4, 036 01 Martin, Slovakia; [2]Institute of Measurement Science SAS, Dubravska cesta 9, 841 04 Bratislava, Slovakia; [3]European Molecular Biology Laboratory, Notkestrasse 85 22607 Hamburg, Germany; [4]ISIS Neutron and Muon Source, UG10, R3, STFC Rutherford Appleton Laboratory, Harwell Campus, Didcot, OX11 0QX, United Kingdom; [5]Institute of Physics, Faculty of Science PJSU, Park Angelinum 9, 040 01 Kosice, Slovakia

*oliver.strbak@centrum.cz

Disrupted iron-sulfur (FeS) cluster biogenesis and pathological iron accumulation associated with the mineralization of biogenic iron-oxide (FeO) nanoparticles are recurrent hallmarks of neurodegenerative disorders [1]. However, their causal interdependence remains unresolved. Our previous hypothesis links impaired FeS cluster synthesis to mitochondrial iron overload and subsequent mineralization of FeO particles (nanograves), with ferritin as a precursor [2]. Here, we critically re-evaluate this relationship using a rotenone (ROT)-induced cellular model of dopaminergic neurodegeneration.

SH-SY5Y neuroblastoma cells were exposed to increasing concentrations of ROT, a mitochondrial complex I inhibitor known to impair FeS-containing enzymes, elevate reactive oxygen species, and enhance mitochondrial iron uptake [3]. In addition, external Fe^{2+}/Fe^{3+} sources were used. Cellular responses were followed over different time scales using a multimodal analytical approach combining cell viability and metabolic assays (MTT test, NMR spectrometry), confocal microscopy of mitochondrial networks, proteomic profiling of FeS assembly machinery, and direct physical characterization of iron accumulation using fluorescent microscopy, SQUID magnetometry, small-angle X-ray scattering (SAXS), MRI relaxometry, XPS, and EPR spectroscopy.

ROT treatment induced a pronounced neurodegenerative phenotype characterized by reduced metabolic activity, changes in mitochondrial morphology, and altered levels of key proteins from the Iron-Sulfur Cluster Assembly Machinery (ISCAM). Concomitantly, intracellular and mitochondrial iron uptake was significantly increased, particularly in the presence of external iron sources (**Figure 1**). However, no clear evidence for nanoscale FeO particle mineralization was detected within the investigated concentration and time ranges. This suggests that iron overload precedes, but does not necessarily culminate in, FeO nanoparticle formation under early or moderate neurodegenerative stress. Additional triggers – such as prolonged inflammation, aging-related redox imbalance, or other specific *in vivo* conditions - are probably required for further FeO mineralization.

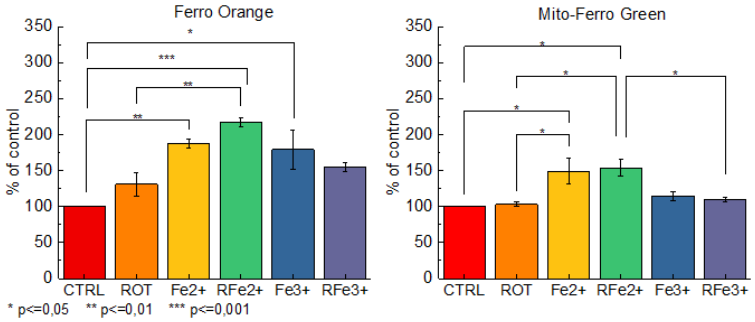

Figure 1: Intracellular and mitochondrial iron uptake.

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STUDIES ON APO-CYSTEIN DESULFURASE AND HOLO-FUMARATE NITRATE REDUCTASE AIMING TO DESIGN ANTIMICROBIAL LIGANDS TARGETING FE-S CLUSTERS

Amalia Stefaniu[1,2,*], Misu Moscovici[1,2], Maria Petrescu[1,2], Roxana Stoica[1,2], Gabriela Săvoiu[1,2], Andrei-Alexandru Muntean[2,3,4], Simin Aysel Florescu[4,5], Maria Manuela Nica[4,5], Costin-Ioan Popescu[6], Andra-Elena Cosoreanu[6], Nicoleta Rusu[1,2]

[1] *National Institute for Chemical Pharmaceutical Research and Development, Bucharest, Romania;*
[2] *Microbial Resource Research Infrastructure — European Research Infrastructure Consortium, MIRRI-ERIC-Romania;*
[3] *"Cantacuzino" National Medico-Military Institute for Research and Development, Romania;*
[4] *Carol Davila University of Medicine and Pharmacy, Bucharest, Romania;*
[5] *Clinical Hospital for Infectious and Tropical Diseases "Victor Babeş", Bucharest, Romania;*
[6] *Institute of Biochemistry of the Romanian Academy, Bucharest, Romania*

[*] *E-mail of presenting author: director-general@ncpri.ro ; astefaniu@gmail.com*

Our studies were focused on three clinically isolated multi-antibiotic-resistant microorganisms: *S. aure*us MRSA GT4853, *E. coli* ESBL HS 9645, and HS 5124. The UV spectra of lysate solutions after adapted cultivations of the wild strains showed characteristic profiles of FeS proteins, but the chromatographic separation of the discovered ones was hardly possible. EPR and CD in UV/VIS studies showed weak signals due to their low concentration, compared to other contaminating proteins.

Consequently, two FeS proteins considered targets in new antibiotic design, the apo-cystein desulfurase (SaSufS), and the holo-fumarate nitrate reductase (FNR) were selected, and their presence and predictive structures were determined by DNA sequencing (Oxford Nanopore Technology, Minion FLO-MIN114 (R10 technology), Rapid Barcoding Kit 24 v14). Basecalling and assembly was done using ONT Dorado software and an autocycler-based multi-assembler protocol. The *bla*TEM, and *bla*CTX-M-15 resistance genes were confirmed for *E. coli* ESBL HS 9645 and HS 5124, respectively.

The responsible productive genes of those FeS proteins led to a similar structure of *S. aureus* PDB ID 8D8S [1] and an identical one to *E. coli* PDB ID 8QTO [2], allowing drug-design studies accordingly. The genes were cloned into *E. coli* BL-21(DE). Their expression is in optimization progress; the purified proteins will serve as substrates for the designed ligands.

The binding affinity of different potential ligands reported previously [3] was assessed using a molecular docking approach on SufS from *Staphylococcus aureus*, PDB ID 8D8S, and on the Escherichia *coli* fumarate and nitrate reduction (FNR) regulator, PDB ID 8QTO. The compounds showing the best docking score were selected, and possible pathways for their synthesis were identified.

Acknowledgments:
This work was carried out through the "Nucleu" Program, Grant number 1N/2023, PN 23-28 "Advanced multidisciplinary researches for the identification and characterization of alternative innovative products and technologies, applicable in the life sciences, BioChemLife", within the National Plan for Research and Development and Innovation 2022-2027, carried out with the support of MCID, project no. PN 23-28 01 02.

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MULTITARGET-DIRECTED LIGANDS POTENTIALLY TARGETING FERROPTOSIS PATHWAYS AND SIMULTANEOUSLY THE HISTAMINE H_3 RECEPTOR

Mihajlo Gajić[1], Elias Böttger[1], Janine Beccu[1], Aleksandra Živković[1], Carsten Berndt[2], Holger Stark[1]

[1] Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitaetsstr. 1, Düsseldorf, Germany; [2] Department of Neurology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine University, Moorenstr. 5, Düsseldorf 40225, Germany

stark@hhu.de

Ferroptosis is an iron-dependent, non-apoptotic form of regulated cell death driven by lipid peroxidation and has been increasingly implicated in the pathogenesis of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and multiple sclerosis [1,2]. In parallel, the histamine H_3 receptor (H_3R), a presynaptic auto- and heteroreceptor predominantly expressed in the central nervous system, modulates the release of multiple neurotransmitters and plays a key role in the regulation of wakefulness, satiety, cognition, and neuroprotection [3]. Despite their individual therapeutic relevance, potential pharmacological interplay between H_3R modulation and ferroptosis-related pathways remains largely unexplored.

Given the shared involvement of both mechanisms in neurodegenerative pathology, simultaneous H_3R antagonism and ferroptosis inhibition may represent a complementary and potentially synergistic therapeutic strategy. The aim of this study was to explore a multitarget-directed ligand (MTDL) approach by integrating pharmacophores associated with H_3R antagonism and ferroptosis inhibition into a single molecular framework.

Using a knowledge-based design strategy, structural fragments of Pitolisant, the only selective H_3R inverse agonist approved for clinical use [3], were combined with fragments derived from the recently reported potent ferroptosis inhibitor UAMC-4821 [2] (Fig. 1). A focused series of hybrid compounds was synthesized to enable preliminary assessment of dual target engagement.

This work establishes a rational structural approach on the development of dual-acting H_3R-ferroptosis modulators and provides a proof-of-concept framework for further pharmacological evaluation. Ongoing studies aim to characterize the biological activity of these hybrids and to assess their potential relevance for neurodegenerative disease intervention.

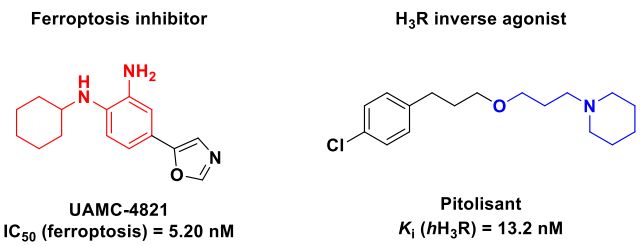

Figure 1. Fragment-based design of dual-target H_3R-ferroptosis ligands. Structural elements from Pitolisant (blue) and UAMC-4821 (red) were combined to generate the MTDL series.

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THE ROLE OF THE DNDC PROTEIN IN DNA PHOSPHOROTHIOATION

Silke Leimkuehler, Ishrat Jahan, Jason Crack, Nicl LeBrun
Institute of Biochemistry and Biology, Department of Molecular Enzymology, University of Potsdam D-14476 Potsdam Germany sleim@uni-potsdam.de +49-331-977-5128 +49-331-977-5603. [2]Centre for Molecular and Structural Biochemistry, School of Chemistry, Pharmacy and Pharmacology, University of East Anglia Norwich Research Park Norwich NR4 7TJ UK N.Le-brun@uea.ac.uk

Recently, we characterized a novel *Shewanella* strain isolated from sediments of the Indian Ocean. A 16S rRNA analysis indicated that it belongs to the species *Shewanella decolorationis*. It was named *Shewanella decolorationis* LDS1. This strain presented an unusual ability to grow efficiently at temperatures from 24°C to 40°C in addition to a high cromate resistance without apparent modifications of its metabolism. Moreover, The analysis of genome sequence showed, that *S. decolorationis* LDS1 possesses the phosphorothioate modification machinery that has been described as participating in survival against various abiotic stresses by protecting DNA.
DNA Phosphorothioation (PT) involves the replacement of a nonbridging phosphate oxygen on the DNA backbone with sulfur. The incorporation of sulfur in this manner endows the PT-modified DNA with altered redox and nucleophilic properties providing it a special ability to be involved in complicated biological functions, such as antioxidation, restriction modification, virus defense, gene transcriptional control and the maintenance of cellular redox homeostasis This oxygen–sulfur swap has been identified to be catalyzed by the gene products of *dndABCDE*. Together with the *dndFGH* gene products present in some bacteria, this system constitutes a defence barrier that can distinguish and attack non-phosphorothioate-modified foreign DNA, resembling DNA methylation-based restriction-modification systems. A rough picture can be drawn to outline the DNA phosphorothioate modification pathway. First, L-cysteine is used as a substrate to generate a persulfide group on the active site cysteine residue of DndA, which is a L-cysteine desulfurase that shares homologies to IscS. The terminal sulfur is next transferred to DndC to make a new persulfide group. The Dnd proteins likely form a complex and once they bind the DNA consensus sequence, the target phosphodiester is likely nicked by DndD in the presence of ATP, and the nicked DNA strands are recognized and stabilized by DndE. The nicking sites might then be activated by adenylation by DndC, followed by nucleophilic attack by the persulphide group of DndC to generate a phosphorothioate linkage.
We characterized DndC in more detail. DndC will was purified under anaerobic conditions and the nature of the Fe-S cluster was investigated by EPR spectroscopy to show a 4Fe-4S cluster. Additionally, the Fe-S cluster on DndC was reconstituted to increase its loading. By site directed mutagenesis plan to determine the cysteine in DndC that is involved in the formation of the persulfide group. To analyze the formation of a persulfide group on DndC, the protein was incubated either with the purified IscS protein from *Shewanella decolorationis* or with the *E. coli* IscS protein. The formation of a persulfide group in DndC will be investigated by mass spectrometry, as established before for various persulfide-containing proteins by our group (Ref). DndC was shown to have ATP pyrophosphatase activity and additionally has a pseudouridine synthase and archaeosine transglycosylase (PUA) domain that is a highly conserved RNA binding domain. It also possess a highly conserved phosphoadenylyl sulfate (PAPS) reductase domain. Competition assay showed that the PAPS activity is more important that the SAM cleavage activity of the protein. At the moment, we exclude that the protein is a radical SAM protein and investigate the PAPS activity for sulfurtransfer in more detail.

Fe–S Clusters as a Molecular mediators between Metabolism and Immunity

Merita Rumano1[1,2], Enkleva Shpati2[3], Elvisa Rumano3[4], Refat Hito4[5]
[1]Biology Department, Faculty of Natural Sciences, University of Tirana, [2]Department of Basic Sciences, Faculty of Medicine, University of Medicine, Tirana, [3]Department of Pharmacy, Faculty of Medical Sciences, Albanian University [4]Pharmacist [5]Student, Faculty of Medicine, University of Medicine, Tirana

merita.rumano@fshn.edu.al

Current evidence suggests that iron–sulfur (Fe-S) clusters contribute to the regulation of innate immune responses during viral infection by modulating host proteins involved in antiviral defense and viral replication [1]. Beyond their emerging roles in antiviral defense, iron–sulfur clusters are essential cofactors that regulate cellular processes central to immune cell function, including DNA repair, transcriptional control, and mitochondrial metabolism. Recent work highlights Fe–S clusters as redox and metabolite sensitive regulators that shape innate immune signaling, host pathogen interactions, and viral pathogenesis, thereby linking immune responses to cellular metabolic state [2]. Consistent with their ancient origin as redox active cofactors, Fe–S clusters have evolved into sensitive cellular sensors of iron, oxygen, and oxidative stress, properties that are directly relevant to immune activation in an oxygen rich and inflammatory environment. In immune cells, the intrinsic redox lability of Fe–S clusters enable dynamic regulation of metabolic and transcriptional programs, such as those mediated by IRP1 during macrophage activation, thereby coupling environmental stress signals to innate immune responses [3, 4, 5]. Mechanistically, the functional versatility of Fe-S clusters derives from their evolutionarily conserved, enzyme assisted biosynthesis, which relies on cysteine desulfurases and dedicated scaffold proteins to assemble and transfer Fe–S cofactors to target proteins. This tightly regulated biogenetic process is highly sensitive to cellular redox, iron availability, and inflammatory stress. As a result, perturbations during immune activation are likely to directly impact Fe–S–dependent metabolic and signaling pathways in innate immunity [6, 7, 5].

As a conclusion, we can mention that the stability and regulated assembly of iron–sulfur clusters are critical for coordinating metabolic and transcriptional programs in immune cells, positioning these cofactors as key determinants of innate immune responses and viral pathogenesis.

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EXPRESSION OF CHLOROPLASTIC IRON-SULFUR ENZYMES IN MITOCHONDRIA OF THE GREEN MICROALGA CHLAMYDOMONAS REINHARDTII

Yanis Aoudache[1,2], Sofia Inturri[1,2], Nicolas Rouhier[2], Claire Remacle[1]

[1] *Genetics and physiology of microalgae, InBios/Phytosystems Research Unit, University of Liege, Belgium,* [2] *Interactions Arbres-Microorganismes, Université de Lorraine-INRAE, France*

c.remacle@uliege.be

Chlamydomonas harbors a flexible metabolic network enabling adaptation to fluctuating environmental conditions. In anaerobic metabolism, ferredoxin 1 (FDX1) and pyruvate-ferredoxin oxidoreductase (PFR1) play central roles, for the formation of acetyl-CoA from pyruvate [1]. Using a synthetic biology approach, we aimed at relocating FDX1 and PFR1 to mitochondria and assessing the metabolic consequences. Modular cloning [2] was used to assemble promoters and terminators to the sequences encoding *FDX1* and *PFR1* in which the chloroplastic targeting sequence was exchanged for a mitochondrial targeting sequence. In the case of FDX1, a nanoluciferase (nanoluc) reporter was added at the N- or C-terminus. These constructs were expressed in a *srta* strain, bearing a mutation in the Sir2-type histone deacetylase (*SRTA*) gene that allows enhanced transgene expression [3]. For both FDX1 fusions, the nanoluciferase activity in transformant cells was relatively weak compared to cells expressing an isolated nanoluciferase. This suggested a lower expression level, possibly due to toxic effects or to an incorrect Fe-S cluster maturation. In parallel, measuring FDX1 activity using purified recombinant proteins showed that untagged FDX1 was more active than the nanoluc-FDX1 fusion while the FDX1-nanoluc fusion was completely inactive, despite *Escherichia coli* cells were able to insert an Fe-S cluster in all proteins. Based on these results, untagged versions of both FDX1 and PFR1 were transformed in a Δ*pfr1*/*Δsrta* strain lacking the endogenous chloroplastic PFR1. Two clones expressing PFR1 in mitochondria have been obtained, in which we need to also confirm the mitochondrial expression of FDX1. In addition, functional assays are underway to determine whether the relocated enzymes possess catalytic activity and to evaluate their impact on mitochondrial redox metabolism. These findings represent a first step toward reconstituting a ferredoxin-dependent anaerobic pathway in Chlamydomonas mitochondria, laying foundation for future metabolic engineering strategies.

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A NEW FAMILY OF FE/S CONTAINING ENZYMES FROM SULFATE REDUCING BACTERIA

Cíntia Carreira[a], Marta S. P. Carepo[b], Corinne Aubert[c], Smilja Todorovic[d], Sofia R. Pauleta[a,*]

[a] *Microbial Stress Lab, UCIBIO – Applied Molecular Biosciences Unit, i4HB, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal.*

[b]*LAQV, REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal, and Universidade Lusofona.*

[c]*Aix Marseille Université, CNRS, LCB UMR 7283, 13402 Marseille, France.*

[d]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal.*

sofia.pauleta@fct.unl.pt

The Orange Protein operon encodes several metalloproteins putatively involved in the cell division of sulfate reducing bacteria and senses redox state of the cell.[1,2] The Orange protein from *Desulfovibrio vulgaris* Hildenborough forms a physiologic protein complex *in vivo* with five conserved proteins from the same gene cluster.[1] The core complex is composed by 12kDa Orp [3-5], and two 32 kDa ATPases (Orp2/Orp3), containing Fe/S clusters. These two proteins are highly homologous and contain MinD-like domains, such as the ones observed in proteins that participate in *E. coli* cell division. Besides this core group of proteins, the other two proteins also contain Fe/S clusters: a small protein, Orp5,[6] and Orp9, a larger protein that belongs to the Mrp/NMP35 family [7] and is proposed to be involved in Fe/S cluster biogenesis.

Here, we present the biochemical, spectroscopic, and electrochemical characterization of the core ORP complex. The results indicate the presence of 4 x [4Fe-4S]. A redox potential of -400 mV ± 5 mV was determined by cyclic voltammetry and potentiometric titration. Moreover, the ability to hydrolyze nucleotides was tested, showing that the complex can hydrolyze ATP. From the ATP concentration dependence study, the following kinetics parameters were determined: K_M = 0.31 ± 0.12 mM and V_{max} = 221 ± 5 pmol min^{-1} mg^{-1}.

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We thank FCT for financial support to SRP (FCT-ANR/BBB-MET/0023/2012), Pessoa Program 2020 and CostAction FeSImmChemNet.

INVESTIGATING THE IMPORTANCE OF *M. AVIUM* AND ALVEOLAR MACROPHAGE IRON HANDLING IN THE CONTEXT OF CHRONIC LUNG DISEASE

Hannah Lynch[1], Patrick Mitchell[2], Seamas C. Donnelly[1,2], Suzanne M. Cloonan[1,3], Claire Healy[1]

1. School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland
2. Tallaght University Hospital Dublin, Ireland
3. Division of Pulmonary and Critical Care Medicine, Weill Cornell Medicine, NY, USA

lynchh2@tcd.ie

Nontuberculous mycobacteria (NTM) comprise a diverse group of environmental mycobacteria, distinct from *Mycobacterium tuberculosis (M. tuberculosis)* and *M. leprae*. They are opportunistic pathogens that cause chronic pulmonary infections, especially in individuals with underlying lung diseases, such as COPD, bronchiectasis, cystic fibrosis and asthma. A characteristic feature of these chronic lung conditions is iron dysregulation, with alveolar macrophages accumulating excess intracellular iron — creating a niche that may favour intracellular pathogens, like *M. avium*.

This study tests the hypothesis that *M. avium* exploits macrophage iron stores to enhance its intracellular replication. Using murine foetal liver-derived alveolar macrophages (FLAM) and primary human alveolar macrophages as *in vitro* models for infection, it was shown that treatment with the iron chelators, deferiprone, deferoxamine or 2,2'-bipyridyl (BPY) significantly reduces *M. avium* intramacrophage replication, while iron supplementation promotes bacterial replication, all without compromising cell viability in culture.

Under defined iron-limited and iron-replete conditions, the iron-associated genes **bfr** and **bfd**, which are homologous to well-characterised iron storage and transport genes in *M. tuberculosis*, exhibit temporally dynamic and iron-responsive regulation, indicating coordinated bacterial strategies to balance iron acquisition and storage, according to environmental availability.

These findings underscore the critical role of accessible intracellular iron pools in supporting *M. avium* persistence, and highlight iron chelation as a promising adjunctive therapeutic approach. This work advances our understanding of how altered iron metabolism contributes to NTM pathogenesis and opens avenues for host-directed therapies in chronic mycobacterial lung infections.

STRUCTURAL INVESTIGATION OF FDX2 PATHOGENIC VARIANTS REVEALS MOLECULAR MECHANISMS DRIVING THE RARE MITOCHONDRIAL DISORDER MEOAL

Grifagni D.[a1,a2#], Doni D.[b#], Cavion F.[b], Buchignani B.[c], Battini R.[d], Baschiera E [e], Desbats M.A.[e], De Pascale E.[b], Boarolo A.[b], Sartori G.[f], Stehling O.[g], Lill R.[g], Santorelli F.M.[h], Salviati L[e], Costantini P.[b*], Ciofi-Baffoni S.[a1*]

[a1] Magnetic Resonance Center and Department of Chemistry, University of Florence, 50019 Florence, Italy, [a2] Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Université Paris-Saclay, 91198 Gif-sur-Yvette, France., [b] Department of Biology, University of Padova, 35121, Padova, Italy, [c] Department of Translational Research and of New Surgical and Medical Technologies, University of Pisa,56126 Pisa, Italy, [d] Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy, [e] Istituto di Ricerca Pediatrica (IRP) Città della Speranza, 35127 Padova, Italy, [f] Department of Biomedical Sciences, University of Padova, 35131 Padova, Italy, [g] Institut für Zytobiologie im Zentrum für Synthetische Mikrobiologie SynMikro, Philipps-Universität Marburg, D-35032 Marburg, Germany, [h] Neurobiology and Molecular Medicine Unit, IRCCS Fondazione Stella Maris, 56128 Pisa, Italy

deborah.grifagni@cnrs.fr

Episodic mitochondrial myopathy with or without optic atrophy and reversible leukoencephalopathy (**MEOAL**, OMIM: 251900) is a rare autosomal recessive neuromuscular disorder characterized by childhood-onset muscle weakness and exercise intolerance. It is caused by mutations in the ferredoxin (*FDX2)* gene, encoding the mitochondrial **FDX2**, a key electron donor for the biogenesis of [2Fe-2S] and [4Fe-4S] iron-sulfur clusters, essential cofactors for mitochondrial enzymes involved in oxidative phosphorylation and metabolic regulation. To date, three pathogenic *FDX2* mutations have been identified:

• **c.1A>T**, affecting the start codon and linked to recurrent myoglobinuria and lactic acidosis;

• **c.431C>T (p.P144L)**, associated with optic atrophy, progressive myopathy, and axonal polyneuropathy;

• **c.200+4A>G**, a novel splicing variant found in a pediatric patient with neuromuscular symptoms.

We employed a multidisciplinary approach combining biochemical, biophysical, and cellular analyses to investigate the molecular consequences of these variants. Structural and dynamic analysis of the **P144L** FDX2 mutant by NMR revealed conformational changes that impair interaction with its partner ferredoxin reductase (FDXR) and disrupt the electron transfer required for iron-sulfur cluster assembly.

In parallel, cellular studies on patient-derived cells carrying the **c.200+4A>G** variant revealed a splicing defect leading to exon 2 skipping and a marked reduction in FDX2 protein levels. A detailed structural characterization of the resulting alternatively spliced protein revealed that, despite being produced in very low amounts, the protein retains functionality. Nevertheless, the low expression levels of this variant are insufficient to support mitochondrial homeostasis, as evidenced by impaired mitochondrial respiration, increased mitochondrial iron accumulation and ROS production, and a marked downregulation of mitochondrial superoxide dismutase 2 (SOD2).

Overall, this study provides molecular insight into how *FDX2* mutations contribute to mitochondrial dysfunction in MEOAL, offering new perspectives for diagnosis and potential therapeutic strategies.

STUDY OF IRON-SULFUR NEET PROTEINS IN BREAST CANCERS

Orane Benoît[a], Julia Kurowski[a], Baptiste Lagardère[a], Enora Festoc[a], Alessia De Santis[a], Thierry Dubois[b], Marie-Pierre Golinelli[a*]
[a]*Institute of Chemistry of Natural Substances – CNRS UPR2301, Paris-Saclay University, Gif-sur-Yvette, France,* [b]*Translational Research Department, Curie Institute – CNRS UMR144, Paris, France*

* *marie-pierre.golinelli@cnrs.fr*

Iron-sulfur cluster proteins are widely distributed across the cell and play crucial roles in numerous biological processes[1]. They are often essential for cellular survival. The NEET family, comprising mitoNEET (CISD1), CISD2 (NAF-1), and CISD3 (MiNT) in humans, consists of [2Fe-2S] cluster-containing proteins localized either at the mitochondria (CISD1 and CISD3) or at the contact sites between the endoplasmic reticulum (ER) and mitochondria (CISD2). Although their physiological and molecular functions remain not fully elucidated, these proteins are thought to be involved in the regulation of iron, calcium, and ROS homeostasis, as well as cell fate processes such as ferroptosis, apoptosis, and autophagy[2]. Due to the presence of the Fe-S cluster, they potentially act as redox regulators in the cell. Studies suggest that NEET proteins are implicated in neurodegenerative diseases, diabetes, and cancers, with overexpression observed in various cancers (gastric, pancreatic, liver, breast, prostate…), where they are thought to promote cancer cell proliferation and support tumor growth[3,4].

Breast cancer is the most common cancer among women. Despite the availability of effective and diverse treatments on the market, the high heterogeneity between subtypes highlights the need for more precise therapies. Five major subtypes are described in the literature[5]: Luminal A (LA; positive for oestrogen and progesterone receptors), Luminal B (LB; positive for oestrogen and progesterone receptors, and sometimes for HER2 receptor), HER2 (positive for HER2 receptor) and Triple-negative A and B (negative for the three receptors). These subtypes have distinct features and therefore respond differently to treatments. Moreover, CISD1 and CISD2 have been reported as mainly overexpressed in Triple-negative breast cancer subtypes. CISD3 is the least studied of the three, although its overall expression in breast cancer seems to be increased[6]. Notably no study to date has compared the NEET expressions in the different subtypes.

Using a wide range of breast cancer cell lines representing the different subtypes as well as healthy breast cell lines, we compared the expression of the three NEET proteins at the RNA and protein levels. We identified a subgroup of breast cancer overexpressing one of the three NEET proteins. We then characterized the effects of this overexpression on the properties of the cancer cell.

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BIOCHEMICAL STUDIES OF ADENINE DNA GLYCOSYLASE (MUTY) FROM *DEINOCOCCUS RADIODURANS*

Shakiba Gholami[1], Célia V. Romão[2], Elin Moe[1]
[1] ITQB-NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal; [2] Departamento Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

E-mail of presenting author: shakiba.gholami@itqb.unl.pt

Deinococcus radiodurans (*D. radiodurans*) is famous for its resistance to radiation and desiccation. Several mechanisms are responsible for this resistance, including DNA repair and proteome protection [1]. The Base excision repair (BER) pathway is one of the repair mechanisms that are essential for cell survival. A/G specific DNA glycosylase (MutY) is a monofunctional DNA glycosylase that plays an important role in BER. The enzyme belongs to the HhH family of DNA glycosylases and removes Adenine and Guanine paired with the oxidation damaged Guanine (8oxoG). Structurally it consists of two domains connected with a long linker, one catalytic domain that possesses the HhH motif and a [4Fe-4S] cluster, and one C-terminal MutT (**8-oxo-dGTP diphosphatase**) like domain. The structure has been determined of full-length *B.acillus stearothermophiles* MutY [2] in complex with DNA, as well as the individual domains of *E.coli* MutY [3]. These studies revealed that the catalytic domain is responsible for recognition of the mismatched adenine nucleotide, while the C-terminal domain interacts with 8oxoG on the opposite strand [2, 4]. Previous studies have shown that many DNA glycosylases from *D. radiodurans* have been structurally modified to increase the enzymes' catalytic efficiency and/or broaden their substrate specificity [5]. These modifications are proposed to increase the organism's DNA repair capacity under genotoxic stress. *D. radiodurans* MutY (DrMutY) has also been studied previously and demonstrated adenine glycosylase activity towards A:G, A:C and A:8oxoG base pairs [6]. However, the crystal structure has never been determined. The aim of this work was to conduct structural studies of this enzyme and perform biochemical and biophysical characterization of it in the BER pathway. We have successfully expressed and purified the DrMutY. The purified protein presents evidence of an iron-sulfur cluster, as indicated by the characteristic broad absorption band around 400 nm in the UV-Visible spectrum. The biological activity of the protein has been analyzed and the protein has been subjected to crystallization trials. In parallel, we have studied MutY sequences within the *Deinococcus* genus and compared them with homologs from other organisms to identify potential functional and structural differences. Together, the results of these experiments contribute to elucidate the functional role of MutY and its potential link to the extremophilic properties of *D. radiodurans*.

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BIOCHEMICAL CHARACTERIZATION OF FERROCHELATASE FROM *MUS MUSCULUS*

Martim Frazão[1], Alexandra S. Alves[1], Ricardo O. Louro[1]

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*

mm.frazao@campus.fct.unl.pt

Heme is an essential cofactor required for numerous cellular processes, including mitochondrial respiration, oxygen transport, and redox metabolism [1]. The terminal step of heme biosynthesis is catalyzed by ferrochelatase, a mitochondrial enzyme that inserts ferrous iron into protoporphyrin IX [2]. In mammalian systems, ferrochelatase contains a [2Fe–2S] cluster, implicating iron–sulfur cluster biogenesis in the regulation and function of heme synthesis [3]. Despite its important role in cellular metabolism and its clinical relevance, the biochemical requirements for proper ferrochelatase activity remain incompletely understood, as well as the role of the [2Fe-2S] cluster in the protein.

The objective of this study was to express, purify, and biochemically characterize murine ferrochelatase using heterologous expression systems. Recombinant murine ferrochelatase was expressed in *Escherichia coli* using the pCoofy12 expression vector and partially purified using affinity chromatography. The purified enzyme lacked its native iron–sulfur cluster, indicating incomplete cofactor incorporation under the expression conditions employed.

These results highlight the challenges associated with reconstituting fully mature mitochondrial enzymes in bacterial systems and emphasize the importance of cellular context for iron–sulfur cluster assembly.

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MITOSOME-TO-CYTOSOL FE–S CLUSTER TARGETING IN *GIARDIA*

Martin Benda[1], Ronald Malych[1], Filipe Folgosa[2], Tomáš Temešinko[1], Róbert Šuťák[1], Ekaterina Panova[1], Luboš Voleman[1] and Pavel Doležal[1]

Giardia intestinalis is a parasitic metamonad with highly reduced mitochondria, termed mitosomes, that retain only the iron–sulfur (Fe–S) cluster assembly (ISC) pathway. While the early ISC machinery is expected to support cytosolic Fe–S protein maturation, the presence of the late ISC pathway is puzzling because *Giardia* lacks mitochondrial Fe–S client proteins.

Using in vitro reconstitution assays, we show that *Giardia* mitosomes can assemble both 2Fe–2S and 4Fe–4S clusters. Disruption of the late ISC component ISCA2 selectively impairs 4Fe–4S, but not 2Fe–2S, cluster formation. In vivo, ISCA2 deletion leads to iron accumulation in mitosomes and a pronounced reduction in cytosolic 4Fe–4S cluster levels, as measured by EPR spectroscopy. This defect correlates with decreased activity of cytosolic Fe–S-dependent enzymes and altered Fe–S-linked metabolic pathways.

Furthermore, we identify functional specialization among the three cytosolic Fe–S scaffold proteins Nbp35, demonstrating that a single paralog is specifically required for 4Fe–4S cluster delivery to pyruvate:ferredoxin oxidoreductase 2 (PFOR2), but not to the closely related PFOR1.

Together, our findings establish a mitosome-to-cytosol Fe–S assembly axis in *Giardia* and reveal substrate-specific targeting within the cytosolic Fe–S biogenesis machinery.

MOLECULAR IMPACT OF THE GLRX5 MET128LYS MUTATION IN HUMAN CONGENITAL SIDEROBLASTIC ANEMIA

Rosanna Cuccaro[1], Lucia Banci[1,2,3]

[1] Magnetic Resonance Center CERM, University of Florence, Via L. Sacconi 6, 50019 Sesto Fiorentino, Florence, Italy; [2] Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine (CIRMMP), Via L. Sacconi 6, 50019 Sesto Fiorentino, Florence, Italy; [3] Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy.
rosanna.cuccaro@unifi.it

Iron-sulfur (Fe-S) cluster biosynthesis in eukaryotic cells is carried out by specialized protein systems [1]. Among these, the ISC machinery is essential for mitochondrial activity, and pathogenic mutations in its components cause severe human diseases. One such disorder is Congenital Sideroblastic anemia (SIDBA3), which is characterized by defective heme synthesis, iron accumulation, and the presence of ringed sideroblasts in erythroid precursors [2].
Missense mutations in human glutaredoxin 5 (GLRX5), a mitochondrial monothiol glutaredoxin, have been linked to SIDBA3 [3,4] and are known to impair the biogenesis of Fe-S clusters, thereby affecting enzymes involved in mitochondrial respiration, DNA repair, and heme synthesis [5]. GLRX5 coordinates a [2Fe-2S] cluster either as a homodimer or as a heterodimer with BOLA3, in which the cluster bridges the two monomers [6,7]. The holo GLRX5-BOLA3 complex can transfer in vitro its [2Fe-2S] cluster to apo NFU1, promoting the assembly of a [4Fe-4S] cluster [8]. Once loaded, holo NFU1 in turn supports the maturation of [4Fe-4S] proteins, including Complex II of the respiratory chain, mitochondrial aconitase (ACO2), and lipoic acid synthase (LIAS) [9,10].

A compound heterozygous mutation in GLRX5 (p.Cys67Tyr and p.Met128Lys) was identified in a young female patient with SIDBA3 [3]. Studies in patient-derived lymphoblasts showed reduced activity of Fe-S- and heme-dependent enzymes, increased oxidative stress, and disrupted iron homeostasis. While the Cys67Tyr mutation directly disrupts [2Fe-2S] coordination in both GLRX5 homo- and heterocomplexes, the molecular impact of Met128Lys remains unclear. In vitro characterization of M128K is therefore essential to determine whether the mutation affects folding, cluster binding, interaction with BOLA3, or cluster transfer to NFU1.

By combining structural and functional analyses, this study aims to provide a molecular explanation for the patient phenotype, bridging existing clinical observations with mechanistic insights at the molecular level.

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AN UNUSUAL FES CLUSTER COORDINATION ENABLES FERROPTOSIS SENSING

Leonie Thewes[1], Svenja Lorenz[2], Yana Bodnar[3], Tom Ole Bach[3], Manuel Etzkorn[4], Gereon Poschmann[5], Christopher Horst Lillig[3], Marcus Conrad[2], and Carsten Berndt[1]

[1] Department of Neurology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine University, Moorenstr. 5, 40225 Düsseldorf, Germany, [2] Institute for Medical Biochemistry and Molecular Biology, University Medicine, University of Greifswald, Fleischmannstraße 8, 17475 Greifswald, Germany, [3] Institute of Metabolism and Cell Death, Helmholtz Zentrum München, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany, [4] Institute of Physical Biology, Faculty of Mathematics and Natural Sciences, Heinrich-Heine University, Universitätsstr. 1, 40225 Düsseldorf, Germany, [5] Institute for Molecular Medicine, Proteome Research, University Hospital and Medical Faculty, Heinrich-Heine University, Universitätsstr. 1, 40225 Düsseldorf, Germany

berndt@hhu.de

FeS clusters are essential cofactors that are typically coordinated by cysteine or histidine residues of the respective enzyme.

We identified glutaredoxin 2 (GRX2), an oxidoreductase of the thioredoxin family, as an unusual FeS protein that coordinates its cofactor between two monomers using active site cysteines as well as cysteines from non-covalently bound glutathione (GSH) molecules as ligands. These GSH molecules are in constant exchange with the surrounding GSH pool; therefore, the cellular GSH concentration controls the stability of the enzymatically inactive GRX2 dimer.

Under ferroptosis-prone conditions, cellular GSH concentrations decrease, resulting in activation of GRX2.

Active Grx2 protects against ferroptotic cell death by de-glutathionylating glutathione peroxidase 4 (GPX4). Engineered GPX4 mutants that mimic glutathionylation showed that this oxidative modification impairs anti-ferroptotic function, leading to death in both cultured cells and mice. Increased susceptibility towards ferroptosis is based on several structural changes induced by glutathionylation affecting membrane binding of GPX4.

In summary, we show a physiological mechanism that senses and regulates ferroptosis (Fig. 1).

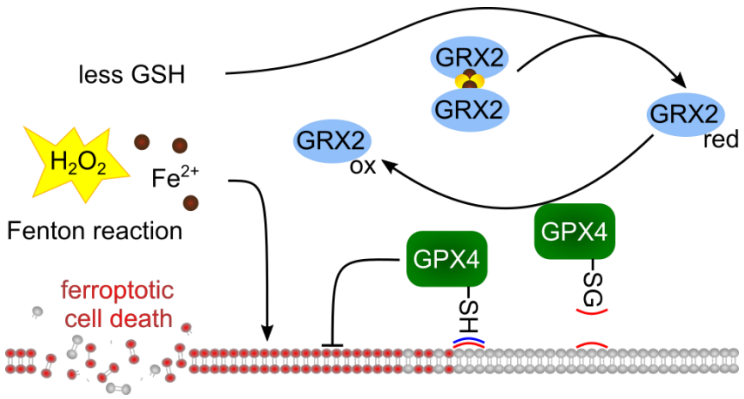


Fig 1. Glutaredoxin 2 in sensing and regulating ferroptosis.

THE FE-S ENZYME ISPH– A SOURCE OF INSPIRATION FOR THE DEVELOPMENT OF NOVEL ANTIBACTERIAL DRUG

Ingrid Span[a], Melissa Jansing[b], Samira Kilian[a], Mara Hebeis[a], Konstantin Bikbaev[a], Chiara Dormann[a], Denise Poire[c], Maria Andrea Mroginski[c]

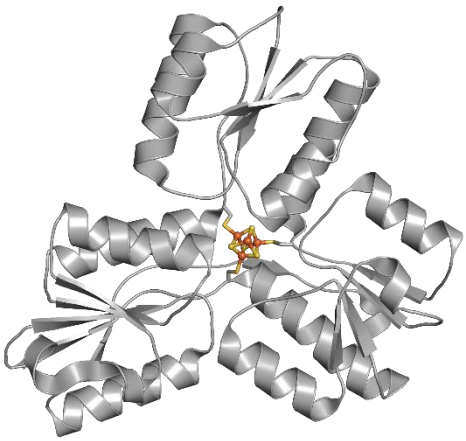
[a] *Bioinorganic Chemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg, Egerlandstr. 1, 91058 Erlangen, Germany;*

[b] *Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany;*

[c] *Department of Chemistry, Technical University of Berlin, Berlin, Germany.*

ingrid.span@fau.de

The increasing number of infections caused by resistant bacteria is a major threat to public health, and there is an urgent need for the development of new classes of antibacterial drugs. A promising approach to overcoming the challenge of emerging resistance is to explore new targets with unprecedented modes of action. Metalloproteins have not been a major focus for the development of antibacterial drugs so far. However, the late members of the DXP pathway are highly promising targets. The iron-sulfur proteins IspG and IspH have been explored in the context of malaria and tuberculosis, with some of the most critical pathogens relying on the DXP pathway, which is not present in humans. Consequently, targeting the late steps of DXP emerges as a highly promising strategy for the development of antibacterial drugs. Our lab focuses on structural studies of the enzyme *E. coli* IspH in complex to small molecules to find new lead structures for drug development.



Crystal structure of *E. coli* IspH with a central [4Fe-4S] cluster in the active site.

NEW CHLOROPHENYL- AND CHLOROPHENOXY-LINKED AZOLE DERIVATIVES: SYNTHESIS, ENZYME MODULATION PROFILES, AND STRUCTURE-BASED INSIGHTS TOWARD NEURODEGENERATIVE TARGETS

Sam Dawbaa[1,2,3], Demokrat Nuha[1,4,*], Asaf Evrim Evren[1], Begüm Nurpelin Sağlik[1], Leyla Yurttaş[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey; [2]Department of Pharmacy, Faculty of Medical Sciences, Thamar University, Dhamar, Yemen; [3]Department of Pharmacy, Faculty of Medical Sciences, Al-Hikma University, Dhamar, Yemen; [4]Faculty of Medical Biochemistry, University for Business and Technology, Prishtina, Kosovo.
demokratnuha@gmail.com , demokrat.nuha@ubt-uni.net

In this work, a set of newly synthesized chlorophenyl- and chlorophenoxy-linked triazole/thiazole derivatives were evaluated for their ability to modulate key enzymes associated with neurodegenerative disorders. Two structural series were examined, comprising hydrazide-based thiazole derivatives (4a–4f) and benzothiazole-derived triazole–thioacetamide hybrids (6a–6c). The compounds were screened for their inhibitory activity toward acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and monoamine oxidase isoforms (MAO-A and MAO-B), enzymes that remain central therapeutic targets in Alzheimer's and Parkinson's disease management [1,2]. Several derivatives demonstrated marked inhibitory potency, with compounds 4e, 6a, and 6b showing the strongest AChE inhibition, consistent with structural features known to enhance cholinesterase binding [3]. In MAO assays, compounds 6a and 6b exhibited pronounced activity against MAO-A, whereas derivatives 4a, 4d, 4f, and 6c showed MAO-A inhibitory values exceeding 55%. Strong MAO-B inhibition was observed particularly for compounds 4a, 4f, 6a, and 6b, highlighting the relevance of the azole–aryl ether scaffold in modulating both enzyme isoforms. Density functional theory (DFT) calculations provided further insight into the electronic properties of the molecules, revealing variations in HOMO–LUMO energy gaps, reactivity indices, and charge distribution patterns consistent with their biological tendencies [4]. Among the most active molecules, compound 4a showed favorable electronic characteristics, whereas compounds 4f and 6a exhibited nucleophilic and electronegative profiles supportive of enzyme interaction. Molecular docking and binding-mode analyses indicated that the benzothiazole moieties preferentially adopted orientations at the peripheral regions of MAO enzymes, while the phenoxy and 4-chlorophenyl fragments occupied sites near the FAD cofactor in the catalytic pocket. The acyl group of both structural series played a crucial role in establishing hydrogen-bond interactions with catalytically relevant residues, in line with established binding patterns for azole-based inhibitors [5].

Overall, the synthesized derivatives demonstrate meaningful inhibitory potential and present valuable structural attributes for further optimization toward neuroprotective drug candidates.

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QM/MM INSIGHT INTO THE DIIRON ACTIVE SITE OF SULERYTHRIN

Maria Andrea Mroginski[a], Samah Moubarak[a]

[a] *Institut für Chemie, Technische Universität Berlin, Straße des 17. Juni 115,10623 Berlin, Germany*

andrea.mroginski@tu-berlin.de

Sulerythrins (SulE) are ferritin-like proteins from aerobic archaea such as Sulfolobus tokodaii.[1] They form domain-swapped dimers and contain binuclear metal centers related to non-heme diiron enzymes involved in oxidative chemistry. Crystal structures of diFe-SulE show small differences in Fe-Fe distances and indications of mixed-valence states, but the chemical identity of the bridging oxygen species has remained unclear.[2,3] To resolve this, we performed hybrid QM/MM calculations on 29 models of the diFe-SulE active site, systematically varying iron oxidation state, Glu95 protonation, and the nature of the bridging dioxo ligand.[4] Our results reveal that diFe-SulE exists as an ensemble of interconverting states: a diferrous center bridged by an end-on di-μ-hydroperoxo ligand, a diferric center stabilized by hydroxo ligands and protonated Glu95, a diferrous di-μ-peroxo-bridged state, also coupled to Glu95 protonation. These states reproduce the experimentally observed Fe-Fe and O-O distances and explain the structural heterogeneity seen in crystallography. The protonation state of Glu95 plays a key role in stabilizing different redox forms and tuning metal-ligand interactions. Thus, QM/MM modeling refines the interpretation of SulE crystal structures and highlights the intrinsic redox and protonation flexibility of its diiron center, features likely essential for peroxide activation and oxidative function in non-heme diiron enzymes.

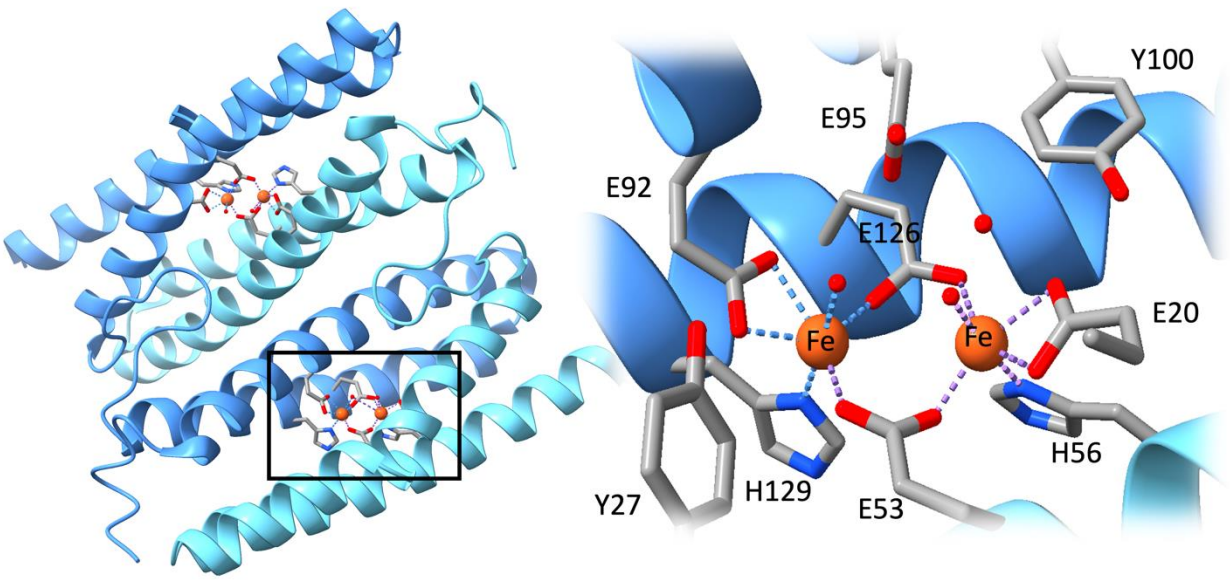

Figure: 3D structure of diFe SulE and zoom of active site (7O8d).

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STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE MITOCHONDRIAL NFU1 FE-S CLUSTER PROTEIN

Martina Masini[1], Jason Crack[2], Nick Le Brun[2], Lucia Banci[1,3,4]

[1] Magnetic Resonance Center CERM, University of Florence, Via L. Sacconi 6, 50019 Sesto Fiorentino, Florence, Italy; [2] School of Chemistry Pharmacy and Pharmacology, Norwich Research Park, NR4 7TJ, Norwich, Norfolk, United Kingdom; [3] Consorzio Interuniversitario Risonanze Magnetiche di Metalloproteine (CIRMMP), Via L. Sacconi 6, 50019 Sesto Fiorentino, Florence, Italy; [4] Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy.

martina.masini1@unifi.it

Iron-sulfur (Fe-S) cluster assembly is essential for mitochondrial metabolism and cellular viability, and defects in this pathway are linked to severe human diseases [1-3]. NFU1 is a mitochondrial protein involved in the maturation and delivery of [4Fe-4S] clusters to specific client enzymes required for energy metabolism [4,5]. Pathogenic mutations in NFU1 cause Multiple Mitochondrial Dysfunction Syndrome 1 (MMDS1), a fatal early-onset disorder characterized by profound metabolic impairment [6,7]. Extensive biochemical and structural studies have highlighted the complexity of mitochondrial Fe-S protein networks and the cooperative interactions that govern cluster trafficking [3,5,8]. Despite its clinical relevance, the molecular basis of NFU1 function and its mechanism of cluster handling remain incompletely understood.

In this work, we investigate the structural and biochemical properties of recombinant human NFU1 with the aim of clarifying its oligomeric behaviour, Fe-S cluster coordination, and interaction with client proteins. Optimized protocols were developed for protein expression, purification, and anaerobic reconstitution of the holo form. Biochemical analyses combined with native mass spectrometry reveal that NFU1 populates dimeric and higher-order oligomeric states in solution. Importantly, the data indicate that individual NFU1 subunits can retain an intact [4Fe-4S] cluster, providing new insight into the relationship between oligomerization and cluster binding. Native MS experiments also support cluster transfer from NFU1 to the client protein lipoic acid synthetase (LIAS), consistent with its proposed physiological role.

These findings contribute to a revised view of NFU1 structural organization and establish a framework for comparative studies of disease-associated NFU1 variants. Ongoing work includes analyses of pathogenic mutants and additional spectroscopic characterization to evaluate structural stability and functional competence. A deeper understanding of NFU1 structure-function relationships is crucial for connecting molecular defects with MMDS1 pathology and for advancing knowledge of mitochondrial Fe-S protein biogenesis.

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THE SUF MACHINERY OF MYCOBACTERIUM TUBERCULOSIS AS A NEW TARGET FOR ANTI-TUBERCULOSIS DRUGS

Mohamad Hamie[a], Christelle Caux[a], Xavier Morelli[b], Sandrine Ollagnier de Choudens[a]

[a] *CNRS, CEA, IRIG, Laboratoire de Chimie et Biologie des Métaux, Univ. Grenoble Alpes, Grenoble, France*; [b] Centre de Recherche en Cancérologie de Marseille, CNRS, INSERM, Univ. Aix-Marseille, France

sandrine.ollagnier@cea.fr

Mycobacterium tuberculosis (Mtb) is a pathogen bacterium that is becoming increasingly resistant to antibiotic treatments, while the variable efficacy of the current vaccine poses a major public health challenge. In this context, identifying new therapeutic targets requires a detailed understanding of the molecular mechanisms involved in *Mtb* pathogenicity.Iron-sulfur (Fe-S) cluster proteins are essential for many cellular processes in *Mtb* [1-2]. Unlike other prokaryotes, *Mtb* has only one Fe-S cluster biosynthesissystem , the SUF machinery [3]. Within this machinery, SufS and SufU ensure the supply and transfer of sulfur required for Fe–S cluster formation **to SufBC_2D [4],** whereas the SufBC_2D complex likely constitutes the central scaffold responsible for the assembly of Fe–S centers prior to their maturation and transfer to apoproteins as studied in *E. coli* [5, 6]. We have two objectives on the Suf system from *Mtb*. i) to characterize the structural and functional properties of the SufBC_2D complex, in order to elucidate the mechanism of Fe-S center biogenesis in *Mtb*. Particular attention is paid to the flavin cofactor. In parallel, an exploratory approach to develop covalent inhibitors (warheads) targeting key residues of the cysteine desulfurase SufS is being implemented, with the aim of identifying new antitubercular agents [7].

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WHEN LIFE PLAYS WITH LEGOS: EXTREME DIVERSITY AND ANCIENT EVOLUTION OF HOMOLOGOUS OXIDOREDUCTASES INVOLVED IN REDOX BALANCE AND ENERGY CONSERVATION

Pierre Simon Garcia[1,2], Valerie De Anda[3,4,5], Brett J. Baker[3,4], Simonetta Gribald[o1], Guillaume Borrel[1]

[1] Department of Microbiology, Unit Evolutionary Biology of the Microbial Cell, Institut Pasteur, Université Paris Cité, Centre National de la Recherche Scientifique UMR 6047, 75015 Paris, France; [2] Laboratoire de Chimie Bactérienne, Institut de Microbiologie de la Méditerranée, Institut Microbiologie Bioénergies et Biotechnologie, Aix-Marseille Université, Centre National de la Recherche Scientifique UMR 7283, 13009 Marseille, France; [3] Department of Marine Science, Marine Science Institute, University of Texas at Austin, TX USA; [4] Department of Integrative Biology, University of Texas at Austin, TX USA; [5] Department of Functional and Evolutionary Ecology, Microbial Oceanography Unit, University of Vienna, Djerassiplatz, 1(UBB), 1030, Vienna, Austria;

pgarcia@imm.cnrs.fr

The regulation of redox balance and energy conservation is fundamental to life. It relies on a large evolutionary network of oxidoreductases containing several metal groups (*e.g.,* [Fe-S], [Ni-Fe], [Ni-Fe-Se] or [Fe-Fe] clusters) and cofactors (*e.g.,* molybdopterin, flavins). They form homologous protein complexes, collectively termed HORBEC (Homologous Oxidoreductase complexes involved in Redox Balance and Energy Conservation). These include hydrogenases, respiratory complex I, and electron bifurcating complexes, central to respiration, fermentation, and methanogenesis. Despite their crucial role, a comprehensive investigation of the diversity and evolutionary history of HORBEC has been lacking. Here, we exhaustively identified and analyzed over 50 protein families representing all HORBEC components across thousands of bacterial and archaeal genomes. We propose a unified nomenclature and classification encompassing 31 complexes, and provide an annotation tool. We highlight the extensive diversity of HORBEC, especially in Archaea. We provide new information on overlooked systems, and identify a new one likely acting as a cation transport platform. We show that HORBEC originated via extensive tinkering of ancestral modules, driven by strong evolutionary constraints. Finally, we infer the presence of respiratory complex I in the Last Universal Common Ancestor, opening questions on its potential role in early energy metabolisms. This work provides an evolutionary framework for HORBEC, representing a fundamental resource to predict and study redox metabolisms of ecological and biotechnological significance.

MITOCHONDRIAL IRON PIRACY: FUELLING MACROPHAGES TO FAIL

Lynne Faherty[1], Ruaraidhrí Jordan[1], Filza Masood[1], Ei Thant Htoo[1], Thomas J. Butler[1], Patrick Mitchell[1], Seamas C. Donnelly[1], Diane M. Ward[3], Natalia Munoz-Wolf[1], Claire Healy[1], Suzanne M. Cloonan[1,2]

1: School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, and Tallaght University Hospital, Dublin, Ireland

2: Division of Pulmonary and Critical Care Medicine, Joan and Sanford I. Weill Cornell Medicine, New York, NY, USA

3: Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, USA

FAHERTYL@tcd.ie

Iron is an essential micronutrient for almost all forms of life. Macrophages are key in the systemic regulation of iron through erythrophagocytosis, and locally at tissues through donating and accepting iron to resolve injury. Macrophages are also key mediators of nutritional immunity, sequestering and diverting iron away from pathogens which seek to steal host metals for their own survival and virulence. This need to sequester iron inertly is in direct contradiction with the iron-demanding nature of macrophage activation, where iron must be mobilized to produce iron-sulfur clusters and heme to fuel mitochondrial bioenergetics and the oxidative response. This becomes more dynamic at mucosal sites like the lung, where resident alveolar macrophages (AMs) face many inhaled pathogens, both intracellular and extracellular, with diverse iron acquisition preferences that must be efficiently neutralised to prevent epithelial damage. *Streptococcus pneumoniae (S.pn*) is a prominent driver of respiratory infection worldwide: while traditionally considered an extracellular pathogen, *S.pn* was recently reported to replicate in splenic macrophages, key in systemic iron handling. Whether this phenomenon occurs in the lung, and the ensuing implications for AM nutritional immunity, are unclear.

In this study, we tested the hypothesis that *S.pn* adopts an intracellular lifestyle within AMs to access host iron, enhancing survival and virulence. We show that *S.pn* replicates intracellularly in primary human (n=23 individual donors) and murine AMs *ex vivo*, heightened in iron-replete conditions (ferric ammonium citrate) and impaired by iron chelation (deferoxamine). Bulk RNA-seq of *S.*pn-infected murine AMs highlighted a suppression of heme biosynthesis, redistributing cellular iron to accumulate in the mitochondrial matrix. This ensuing mitochondrial iron overload is beneficial to *S.pn* and can be targeted both pharmacologically *in vitro* and *ex vivo* with the mitochondrially-active iron chelator deferiprone and genetically *in vivo* in *S.pn* instilled-mice to impair proliferation. Finally, we identify AM mitochondrial iron overload as a phenomenon of clinical relevance in chronic obstructive pulmonary disease (COPD) which promotes susceptibility to recurrent infection and a microbial signature defined by atypical means of iron acquisition. We propose mitochondrial iron as a target of intracellular bacterial iron piracy, exploited by *S.pn* to impair macrophage defences. This phenomenon may extend to other respiratory pathogens in COPD, where high prevalence and persistent colonization are major challenges. Iron dysregulation in the COPD lung could provide both a nutrient advantage and weaken innate defenses, helping explain the disproportionate susceptibility to *S.pn* and other microbes.

Deferiprone leads to the accumulation of specific myeloid progenitor populations in the bone marrow and leads to the reprogramming of macrophage populations

Ruaraidhrí Jordan[1], Lynne Faherty[1], Suzanne M. Cloonan[1,2]

1: School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, and Tallaght University Hospital, Dublin, Ireland

2: Division of Pulmonary and Critical Care Medicine, Joan and Sanford I. Weill Cornell Medicine, New York, NY, USA

jordanr2@tcd.ie

Deferiprone is an EMA/FDA-approved small-molecule iron chelator used to treat iron overload-associated anemic disease, but its systemic use is limited by severe hematological toxicities including neutropenia and agranulocytosis. These side effects lead to a functional loss in critical immune cell populations. These adverse effects have been proposed to arise from off-target copper chelation within the bone marrow, impairing myeloid differentiation. While deferiprone has shown therapeutic promise in the treatment of numerous diseases, including chronic obstructive pulmonary disease, its current side-effect profile makes it unsuitable for use in wider disease [1].

Here, we show that systemic dosing of deferiprone led to worsened outcomes from *in vivo* infection with *Streptococcus pneumoniae.* We also show, by flow cytometry, that systemic deferiprone administration in mice induces an accumulation of myeloid progenitors at, and upstream of, the common myeloid progenitor stage. This is consistent with an early block in myelopoiesis rather than terminal cell loss. While deferiprone treatment reduced bone-marrow iron content as measured by graphite furnace atomic absorption spectroscopy, no corresponding depletion of copper was observed, arguing against copper loss as the primary mechanism.

Notably, we show by seahorse metabolic analysis that bone-marrow-derived macrophages from deferiprone-treated mice exhibited persistent alterations in oxidative phosphorylation and extracellular acidification rate, indicating a persistent energetic alteration in immune cells from deferiprone treated mice. Further, we show that these macrophages demonstrate enhanced pro-inflammatory cytokine expression in response to sterile and bacterial stimuli and improved killing of intracellular *Haemophilus influenzae*. This enhancement was maintained after chelator withdrawal and was not reversed by subsequent iron supplementation.

Together these data indicate durable immunometabolic reprogramming of hematopoietic progeny following transient systemic iron chelation. These findings indicate that deferiprone-associated neutropenia may reflect iron-dependent developmental reprogramming within the bone marrow rather than acute metal deprivation alone. This research provides improved insight into the off-target effects, and highlights potential therapeutic applications, of deferiprone.

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MULTIMERISATION OF IRON-SULFUR CLUSTER PROTEIN HCF101 IN THE HUMAN PARASITE TOXOPLASMA GONDII AND ITS IMPLICATION FOR PROTEIN SYNTHESIS

Maxime Gonzalez-Durany[1], Ambre Maupin[1], Esther Pouzet[1], Yann Bordat[1], Julie Bals[2], Florence Vignols[2] and Sébastien Besteiro[1].

[1] LPHI, Univ. Montpellier, CNRS, INSERM, Montpellier, France; [2] IPSiM, Univ. Montpellier, CNRS, INRAE, Institut Agro, Montpellier, France

Sebastien.besteiro@inserm.fr

Toxoplasma gondii is a protozoan parasite that can potentially cause a disease called toxoplasmosis. Although the infection is typically asymptomatic, it can pose serious risks to immunocompromised individuals, and to developing fetuses in the congenital form of the disease. *T. gondii* is highly prevalent worldwide, as it can infect nearly all warm-blooded vertebrates and is estimated to be present in about one third of the global human population. Transmission most commonly occurs through the ingestion of undercooked meat contaminated with tissue cysts, which contain a persistent form of the parasite.

There are three pathways for Fe-S clusters biogenesis in *T. gondii* [1], which are located within the mitochondrion, in a non-photosynthetic plastid harbored by the parasite [2, 3], and in the cytoplasm. We have recently shown that the *T. gondii* homologue of a protein called HCF101 [4] is essential for parasite viability as part of the CIA (Cytosolic Iron-Sulfur cluster Assembly) pathway, where it likely transfers Fe-S clusters to a widely-conserved regulating factor of protein synthesis called ABCE1. Because there is no HCF101 homologue in mammals, this interaction is unique and offers the prospect of designing specific inhibitors. Thus, we investigated the level of protein organization of TgHCF101 through a structure-function relationship study and we have explored the implications for Fe-S transfer to TgABCE1 and for its essential cellular role.

Our work highlights a lineage-specific adaptation in the interaction between a client and a transfer Fe-S protein that could be exploited to design novel anti-parasitic drugs.

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USING A 'PLUG AND PLAY' NANOCAGE ASSEMBLY PLATFORM TO CONSTRUCT ANTIVIRAL NANODEVICES

Yujie Sheng[a], Yasmin Surani[b], Isobel A. Manterfield[b], Wu-Lu Meritxell[c], Julia Tree[b], Maria Andrea Mroginski[c], Mark Sutton[a,b], Kourosh H. Ebrahimi[a]

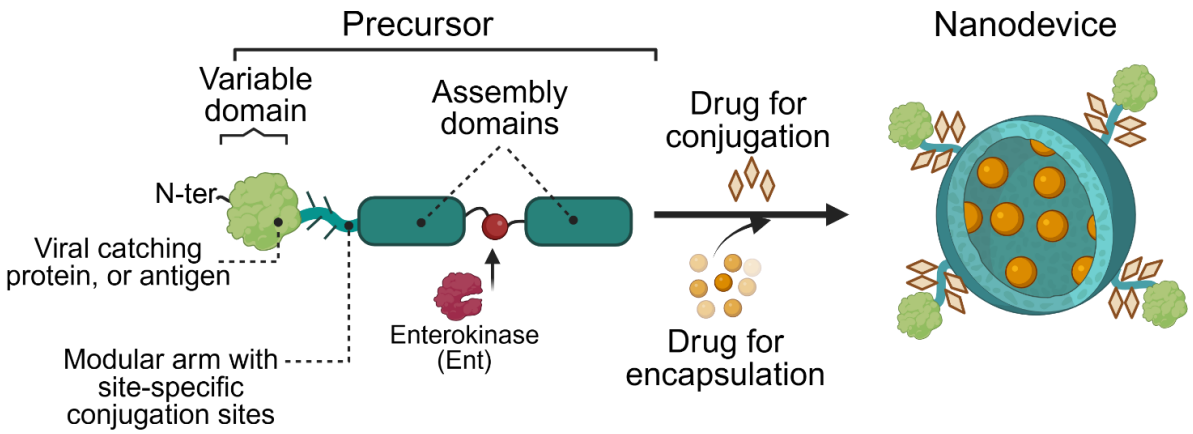
[a] *Institution of Pharmaceutical Science, King's College London, 150 Stamford Street, London.*

[b] *Technology Development Group, UK Health Security Agency, Research and Evaluation, Porton Down, Salisbury SP4 0JG, United Kingdom*

[c] *Institut für Chemie Technische Universität Berlin, PC 14 Straße des 17. Juni 135, Berlin, 10623, Germany*

Yujie.sheng@kcl.ac.uk

Biological nanodevices and molecular machines provide a route to build functional systems at the nanoscale with defined structures and programmable surfaces. Protein nanocages are attractive platforms to engineer for constructional of new bionanodevices, but it is often difficult to control their assembly and access their building blocks for creating components that can be assembled to nanodevices with various applications such as in antiviral therapy and prevention. To address this challenge, we created a 'plug and play' molecular assembly platform.[1] We applied a multidisciplinary approach and used our 'plug and play' platform to construct antiviral nanodevices. We demonstrate designed rules. We describe the creation of nanodevices with a viral catching hand linked to a nanobody with a modular arm that can be used for small molecule conjugations. We demonstrate the effectiveness of the individual building blocks to HIV-1 surface glycoprotein and show that the assembled nanodevice have antiviral activity against influenza A virus at nanomolar range. Our data demonstrate a practical strategy to rapidly construct and develop modular antiviral nanodevices for antiviral prevention and treatment.



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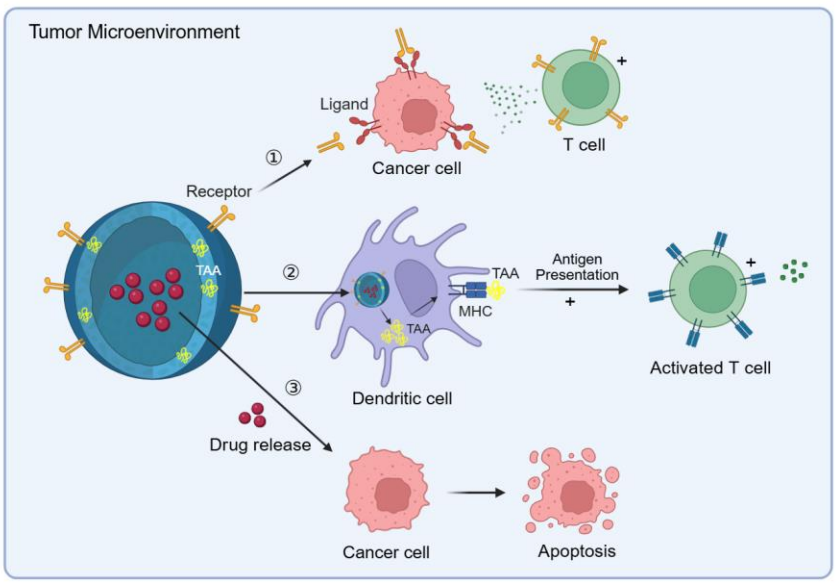
TOWARDS MULTIFUNCTIONAL CHEMO-IMMUNOTHERAPEUTICS USING PLUG & PLAY SYNTHETIC GENE PLATFORM

Shujie Li[1], Kourosh Ebrahimi[1*]
[1] Faculty of Life Science and Medicine, King's College London.
Email: shujie.4.li@kcl.ac.uk

Effective cancer management increasingly depends on integrating different treatments. Immunotherapy has emerged as a promising strategy by using the host immune system,[1] while conventional chemotherapy remains essential despite limitations in systemic toxicity and poor delivery.[2,3] Therefore, combining these distinct approaches offers a powerful strategy to improve efficacy while reducing toxicity. However, developing and constructing multifunctional therapeutics combining immunotherapy and multiple chemotherapies remains a challenge.[4] To address this challenge, we developed a 'plug and play' synthetic gene platform.[5] I demonstrate the use of this platform to design "three-in-one" chemo-immunotherapeutic candidates. I provide preliminary data on the development and characterisation of these chemo-immunotherapeutic candidates. Ultimately, this "plug-and-play" synthetic gene platform is expected to provide a promising strategy for developing novel safe and efficacious cancer chemo-immunotherapeutics.



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ANCIENT MINIMAL PATHWAYS FOR IRON–SULFUR CLUSTER ASSEMBLY FROM THE LAST UNIVERSAL COMMON ANCESTOR

Mohamad Ali Abbas[1], Dussouchaud Macha[2], Dupuy Emile[2], Barras Frédéric[2], Ollagnier De Choudens Sandrine[1]

1 Université Grenoble Alpes, CNRS, CEA, IRIG, Laboratoire de Chimie et Biologie des Métaux, Grenoble, France, 2 Department of Microbiology, Unit Stress Adaptation and Metabolism in Enterobacteria, Institut Pasteur, Université Paris Cité, UMR CNRS 6047, Paris, France

Abbas.mohamadali@Cea.fr

Iron–sulfur (Fe–S) clusters are ancient and ubiquitous protein cofactors that were essential for the emergence of life on the anoxic early Earth [1,2]. While Fe–S clusters are often hypothesized to have formed spontaneously in early anaerobic, iron- and sulfide-rich environments, increasing evidence supports the existence of early enzymatic assembly pathways. Recently two minimal Fe–S biogenesis systems, MIS and SMS, have been proposed to represent ancestral pathways tracing back to the Last Universal Common Ancestor (LUCA) [3] and to have given rise to the modern ISC, NIF, and SUF machineries.

The MIS system consists of IscS- and IscU-like homologs, MisS and MisU, and has been experimentally validated as a functional Fe–S biogenesis pathway [4,5]. The second minimal system, SMS, is composed of homologs of the SUF scaffold proteins SufC and SufB, termed SmsC and SmsB. Studies of the SMS system revealed that the $SmsC_2B_2$ heterotetramer assembles a single [4Fe–4S] cluster unexpectedly on an SmsC subunit rather than on SmsB [6,7]. Structural and sequence analyses resolved the holo-complex architecture and identified a conserved CXnCXXC motif in SmsC as the cluster-binding site [6,7]. *In vivo* complementation assays in *Escherichia coli* lacking ISC and SUF demonstrate that the SMS system from *M. jannaschii, M. thermoacetophila and A. fulgidus* support anaerobic Fe–S biosynthesis using sulfide as a sulfur donor [6,8].

These findings advance our understanding of the origins and diversification of Fe–S biogenesis and raise key questions regarding the physiological relevance of CXnCXXC-mediated cluster assembly in SmsC and the potential functional cooperation between MIS and SMS in organisms harboring both systems. Our comparative studies of these systems in *M. acetivorans* and *M. stadtmanae* aim to address these questions and further elucidate the evolution of early Fe–S biogenesis pathways.

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IDENTIFICATION OF KEY INTERMEDIATES COORDINATING IRON AND SULFUR ASSEMBLY IN THE COURSE OF [2FE-2S] CLUSTER SYNTHESIS

Rémi Mor-Gautier,[a] Maren Hellen Hoock,[b] Giorgio Caserta,[c] Erwan Galardon,[d] Hubert Gorny,[a] Kristian Want,[a] Volker Schünemann,[b] Bénédicte Burlat,[e] Benoit D'Autréaux[a]*

[a] *Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette cedex, France*
[b] *Department of Physics, Biophysics andMedical Physics, University of Kaiserslautern-Landau, Erwin-Schrödinger-Str. 46, 67663 Kaiserslautern, Germany*
[c] *Institut f¨ur Chemie, Technische Universit¨at Berlin, Straße des 17. Juni 135, 10623 Berlin, Germany*
[d] *Laboratoire de Chimie et Biochimie Pharmacologique et Toxicologique, UMR 8601 CNRS, UniversitéParis Descartes, PRES Paris cité, 45 rue des Saints Pères, 75270 Paris Cedex 06, France*
[e] *Aix Marseille Univ, CNRS, Laboratoire de Bioénergétique et Ingénierie des Protéines (BIP), 31 chemin Joseph Aiguier 13402, Marseille, France*

*benoit.dautreaux@i2bc.paris-saclay.fr

Iron-sulfur clusters (Fe-S) are ubiquitous inorganic protein cofactors made of iron and sulfide ions fulfilling numerous biological functions. They are biosynthesized by multiprotein machineries via a highly conserved multistep process.[1] In eukaryotes, their synthesis is carried out by the mitochondrial Iron-Sulfur Cluster (ISC**)** machinery.[2] The core ISC machinery assembles [2Fe-2S] cluster in four main steps: first the insertion of iron into the assembly site of the scaffold protein ISCU2, then the acquisition of a persulfide by transfer from the cysteine desulfurase complex NFS1-ISD11-ACP, which is accelerated by the frataxin protein FXN.[2, 3] The persulfide is reduced into sulfide by the ferredoxin-2 FDX2 and its reductase FDXR, leading to the formation of the [1Fe-1S] precursor and finally a [2Fe-2S] cluster by fusion of [1Fe-1S] precursors upon dimerization of ISCU2.[4] FXN plays a key regulatory role in this process, and a defect in its expression leads to the human disease Friedreich's ataxia.[5] Although this system has been extensively investigated using biochemical approaches and through the determination of several structures, a number of mechanistic aspects remain poorly understood, notably how iron and persulfide supplies, and the generation of sulfide are coordinated into ISCU2. Strikingly, both persulfide transfer and persulfide reduction on ISCU2 require the presence of iron,[3, 4] suggesting that the persulfides in NFS1 and ISCU2 interact with iron in the form of iron-persulfide intermediates to trigger the nucleophilic attack of the cysteine receptor on ISCU2 and to allow the reduction by FDX2. Using an *in vitro* reconstitution of the human ISC machinery and site directed mutagenesis on key cysteine residues of ISCU2, we have isolated intermediates along the trans-persulfidation reaction. Biophysical techniques, including electron paramagnetic resonance (EPR), Mössbauer and nuclear resonance vibrational (NRVS) spectroscopies were used to detect and characterize these intermediates at each stage of persulfide transfer. These analyses reveal that the electronic environment of the iron center is modulated by the presence of persulfide throughout the process, supporting the formation of iron-persulfide intermediates during Fe-S cluster biosynthesis.

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IDENTIFICATION OF ELECTRON DELIVERY ALTERNATIVES FOR ENGINEERED NITROGENASE SYSTEMS

Alessandro Gigli[1], Laura Cutugno[1], Luis M. Rubio[1]

[1] Centro de Biotecnología y Genómica de Plantas (CBGP) - INIA-CSIC, Campus de Montegancedo, Pozuelo de Alarcón, 28223 Madrid, Spain

a.gigli@inia.csic.es

Engineering plants capable of Biological Nitrogen Fixation (BNF) would have a transformative agricultural and environmental impact. This objective comes with major challenges, including the complexity of the key enzymatic machinery, nitrogenase, and its sensitivity to oxygen. To address these challenges, the approach developed by the group of L. Rubio has focused on dividing the nitrogenase pathway into working modules and on the functionalization of these modules in yeast mitochondria as a model, an approach that has already proven successful [1,2].

The objective of this work is the implementation of the energy delivery module, which is composed of the pyruvate:ferredoxin/flavodoxin oxidoreductase (NifJ) and the flavodoxin (NifF). This module channels electrons derived from pyruvate oxidation through three [4Fe–4S] clusters toward the reduction of the FMN cofactor (flavin mononucleotide) of the flavodoxin, which subsequently transfers electrons to the nitrogenase system through NifH. Ultimately, this work aims to generate a library of functional electron-donating modules, enabling future optimization of metabolic pathways supplying reducing power to nitrogenase.

For this reason, we focused on identifying and testing alternatives to NifJ. We developed a novel bioinformatic pipeline based on structural similarity and protein–protein interaction prediction for the identification of alternative enzymes, and we explored different sources of reducing power. The bioinformatic analysis began with available structures of NifJ, NifF, and related members of the PFOR family. Structural gaps in NifJ were filled using AlphaFold v2/v3 and homology modelling. These structures were then used as queries in Foldseek to identify structurally related proteins. Two primary structural hits—a PFOR from *Moorella thermoacetica* and flavodoxin A from *Escherichia coli*—were selected, and additional candidates were identified through literature analysis [3,4]. Docking simulations using ClusPro 2.0 were performed to compare native NifJ–NifF interactions with alternative protein pairs, yielding comparable interaction models. These alternatives are now being tested in vivo, both in E. coli (to assess compatibility between different components) and in yeast.

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EFFECT OF UV-B IRRADIATION ON IRON ACCUMULATION IN *CHLORELLA SOROKINIANA*

Isidora Santrač[1], Milena Dimitrijević[1], Marina Stanić[1], Valentina Ćurić[2], Marija Tanović[1], Ivan Spasojević[1], Jelena Danilović Luković[1]

[1]*University of Belgrade - Institute of Multidisciplinary Research, National Institute of the Republic of Serbia, Belgrade, Serbia*

[2]*Faculty of Biology, University of Belgrade, Belgrade, Serbia*

isidorasantrac@hotmail.com; isantrac@imsi.ac.bg.rs.

Microalgal biomass represents an alternative and sustainable feedstock for aquaculture and animal feed. In addition to supplying energy and proteins, such biomass should also ensure an adequate intake of iron and other essential microelements. However, iron limitation is a common challenge in microalgal cultivation due to the poor solubility and bioavailability of ferric iron (Fe^{3+}) at neutral pH. In this study, we propose a simple and cost-effective method for iron loading of microalgae, aimed at improving the performance of "fortified" feeds. The industrially relevant microalgal species *Chlorella sorokiniana* was exposed to UV-B radiation for 30 or 60 minutes in the presence of high concentrations of $FeCl_3$, followed by a 24 h incubation period. Iron uptake was quantified by Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP-OES), while biomass quality, specifically lipid and protein content, was assessed using Fourier Transform Infrared Spectroscopy (FTIR) to verify that metabolic profile is not adversely affected. ICP-OES analysis revealed a significant enhancement of iron accumulation following UV-B treatment, with the 30 minutes exposure resulting in the highest iron content (82.9±15.4 mg g^{-1} dry biomass) compare to control (36.03±3.7 mg g^{-1} dry biomass). The observed increase is attributed to a dual mechanism: photoreduction of iron, improving its bioavailability, and mild UV-induced alterations of the cell wall or membrane permeability. FTIR analysis indicated structural changes in the cell wall and a slight, metabolic shift, with no adverse effects on lipid or protein content. This approach offers a sustainable strategy for generating iron-rich biomass, and also implicates physiological role of UV irradiation in iron metabolism of microalgae.

THE TOLERANCE OF THE EXTREMOPHILIC MICROALGA *CHLAMYDOMONAS ACIDOPHILA* STRAIN PM01 TO HIGH ENVIRONMENTAL MANGANESE

Isidora Santrač[1], Milena Dimitrijević[1], Milan Žižić[1,2], Marina Stanić[1], Valentina Ćurić[1], Wilfred Hagen[3], Bernd Zechmann[4], Jon Pittman[5],Ivan Spasojević[1,*], Jelena Danilović Luković[1]

[1]*University of Belgrade - Institute of Multidisciplinary Research, National Institute of the Republic of Serbia, Belgrade, Serbia*

[2]*Elettra Sincrotrone Trieste S.C.p.A., Basovizza, Trieste, Italy*

[3]*Department of Biotechnology, Delft University of Technology, Delft, the Netherlands*

[4]*Center for Microscopy and Imaging, Baylor University, Waco, TX, USA*

[5]*Department of Earth and Environmental Sciences, School of Natural Sciences, The University of Manchester, Manchester, UK*

isidorasantrac@hotmail.com; isantrac@imsi.ac.bg.rs.

Chlamydomonas acidophila PM01 is an extremophilic green microalga thriving in acidic, metal-rich environments, such as acid mine drainage ponds, demonstrating remarkable tolerance to heavy metals. Manganese (Mn), a common pollutant that remains soluble across broad pH ranges and is notoriously difficult to remove, becomes toxic in excess through mismetallation of Fe-enzymes and induction of oxidative stress. Herein, using advanced analytical and microscopic methods, we show that *C. acidophila* PM01's adaptation to elevated Mn levels hinges on preventing intracellular Mn accumulation via enhanced vacuolization and excretion, possibly supported by modifications in the cell wall. We found that *C. acidophila* PM01 accumulated only trace amounts of Mn, sequestered in chloroplasts and vacuoles, with excess routed to contractile vacuoles. Mn persisted as Mn^{2+} in a distorted octahedral coordination, exhibiting behavior characteristic of a two-level (S = 1/2) spin system. Exposure to excess Mn^{2+} in microalgae led to increased vacuolization, mitochondrial swelling, and enlargement of starch grains, particularly around the pyrenoid, indicating an energy stress response and strategies to regulate intracellular Mn levels. This did not alter cell shape or induce mucilage release but increased the size of vesicle-like structures and reduced the diameter of cell wall pores, suggesting enhanced cell communication and restricted ion diffusion. *Mn excess caused lipid peroxidation with initial ROS accumulation led to a sustained decrease in thiol levels, indicating irreversible oxidative damage. This pro-oxidative state was associated with diminished levels of iron-containing antioxidant enzymes, likely due to Mn^{2+}-induced mismetallation and competition for metal cofactors, impairing the microalga's antioxidative defense mechanisms. Mn exposure slightly improved photosynthetic performance, suggesting enhanced energy conversion and photochemical efficiency, rather than heightened photoprotection. Though C. acidophila PM01* displays a remarkable tolerance to Mn, achieved by minimizing intracellular accumulation while sustaining oxidative balance and energy demands, its Mn sequestration capacity is limited.